

# **Predictors of response of AIDS-associated Kaposi Sarcoma to standard Chemotherapy**

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Master of Medicine (MMED)  
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**By**

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## **DECLARATION**

I hereby declare that this submission is my own work and it has not been submitted to this or any other universities. All sources and references I have used or quoted have been indicated and acknowledged.

This work was supervised by Dr. A. Mosam (Department of Dermatology)

**Omran A. El-koha**

June 1<sup>st</sup>, 2005

## **Dedication**

To the memory of my

### **Father**

Who instilled in me the value of hard work;

Who was always stressing on the value of education;

From whom I learned honesty and generosity;

The day I said goodbye to him will always be in my mind and heart.

You will be remembered forever.

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## **LIST OF ABBREVIATIONS**

**Hb:** Haemoglobin

**WBCs:** white blood cells

**CD4:** T-helper lymphocytes

**CD8:** Suppressor T lymphocytes

**S. Albumin:** Serum Albumin

**HIV:** Human Immunodeficiency Virus

**KS:** Kaposi Sarcoma

**HAART:** Highly Active Antiretroviral Therapy

**VL:** Viral Load

**ACTG:** Aids Clinical Trial Group

**FBC:** Full Blood Count

**U/E:** Urea/Electrolytes

**LFT:** Liver Function Test

**RNA:** Ribonucleic acid

**HHV8:** Human Herpes Virus 8

**KSHV:** Kaposi Sarcoma Herpes Virus

**ART:** Antiretroviral Therapy

**HPV:** Human Papilloma Virus

**EBV:** Epstein Barr Virus

**PIs:** Protease Inhibitors

**AIDS:** Acquired Immunodeficiency Syndrome



**VEGF:** Vascular endothelial growth factor

**GPCR:** G- protein coupled receptor

**DNA:** Deoxyribonucleic Acid

**PCR:** Polymerase chain reaction

**ORF:** Open Reading Frame

**FLIP:** Flice- inhibitory protein

**Flice:** Fas-associates death domain-Interleukin- beta Converting enzyme

**IRF:** Interferon Regulatory Factor

**FADD:** Fas-activated death domain

**AZT:** azidothymidine

**ABV:** doxorucin, vincrstine and bleomycin

**ROC CURVE:** Receiver Operating Characteristic curve

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## **ABSTRACT**

### **Predictors of response of AIDS-associated Kaposi-Sarcoma to standard chemotherapy**

#### **Overview:**

Kaposi Sarcoma is the most common HIV-associated cancer. Its etiology and pathogenesis is not fully understood. Little is known about what predicts prognosis, survival and therapeutic response in HIV-KS. In South Africa given the high seroprevalence rates of HIV-1 and human herpes virus 8 (HHV 8), Kaposi's sarcoma is a significant problem. The majority of patients have been treated solely with palliation due to the poor outcome associated with a diagnosis of HIV-KS, more so in the absence of highly active antiretroviral therapy (HAART). Since the national ARV rollout programme and the availability and accessibility of HAART to all patients with a diagnosis of HIV-KS, a new strategy has to be established to enable adequate patient selection for chemotherapy. There have been a few published studies addressing the predictors of response to chemotherapy in the first world. However, this is the first study of these factors in HIV-1 infected African patients with Kaposi's sarcoma.

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**Aim:**

To identify and assess the potential value of several parameters predictive of outcome, survival and therapeutic response in HIV- infected patients with KS. Clinical, hematological, biochemical, immunological and virological variables were evaluated.

**Methods:**

We collected data from 25 patients with AIDS-KS who were enrolled in a phase III randomized controlled trial comparing HAART alone with the combination of HAART and chemotherapy. All patients were from the combination therapy arm. The following variables were evaluated as predictors of prognosis and therapeutic response: age, gender, ethnic origin, Haemoglobin (Hb), white blood cells (WBCs), lymphocytes, neutrophils, platelets, S.albumin, ALP, GGT, CD4 count, HIV viral load. These variables were assessed in patients at baseline and month 6 of therapy. Patients were staged into good risk and poor risk according to the AIDS clinical trial group (ACTG) criteria. The outcomes assessed were response to treatment and mortality.

**Results:**

A total of 25 patients participated to the study. Of these 16(64%) were males and 9(36%) were females, with male: female ratio of 2.7:1. Median age was 34 years (24-47); all patients were of Black African origin. Of the 21 patients, 15 (71.4%) were of good prognosis and 6(28.6%) were of poor prognosis.

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At baseline the median values of the different variables were as follows: Hb 10.9 g/dl, WBCs  $5.95 \times 10^9/L$ , lymphocytes  $1.7 \times 10^9/L$ , neutrophils  $3 \times 10^9/L$ , platelets  $272 \times 10^9/L$ , S.albumin 30 g/L, total protein 88 g/L, ALP 64 U/L, and GTT 21 U/L, CD4 count was 255 cells/mm<sup>3</sup>, HIV-RNA viral load was 42000(4.6logs).

At month 6, 22 patients remained alive, their median values were: Hb 12.2 g/dl, WBCs  $4.65 \times 10^9/L$ , lymphocytes  $1.5 \times 10^9/L$ , neutrophils  $3 \times 10^9/L$ , platelets  $301 \times 10^9/L$ , S.albumin 36.5 g/L, total protein 84.5 g/L, ALP 78.5 U/L, GTT 44.5 U/L, CD4 count 288 cells/mm<sup>3</sup>, HIV-RNA viral load was 50500(4.69logs).

The baseline median CD4 and HIV-RNA viral load counts for the 3 patients who died before month 6 were 47 cells/mm<sup>3</sup> and 31000(4.6logs); respectively.

Response to therapy was evaluated in 21(84%) patients as 4(16%) patients were missing, of the 21 patients 3 (14.3%) had complete response and 18(85.7%) had partial response. With respect to sex 2(14.3%) males had complete response and 12(85.7%) had partial response, 1(14.3%) female had complete response and 6 (85.7%) had partial response.

Non-parametric statistics were used because of the small sample size and the skewness of the data. Variables were described using medians and ranges, and compared between two independent groups using Mann-Whitney tests. Baseline and

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month 6 comparisons were done using Wilcoxon signed ranks tests. Receiver Operating Characteristic (ROC) curves were used to analyze cut points to optimize sensitivity and specificity of a quantitative variable for a dichotomous outcome.

## **Discussion**

In the univariate analysis age and sex didn't influence prognosis and therapeutic response, the influence of ethnic origin couldn't be assessed as all patients were of the same ethnic origin. Baseline WBCs ( $P=0.004$ ) and lymphocytes ( $P=0.026$ ) were significantly associated with complete response. Higher values of GGT ( $p=0.001$ ); ALP ( $P=0.006$ ) were associated with more deaths.

Baseline CD4 count and HIV viral load were not of predictive value, although change CD4 ( $P=0.002$ ) and VL ( $p=.000$ ) over time was significant and most likely attributed to response to therapy. 90.9 % of patients reached undetectable HIV-1 Viral loads at month 6.

## **CONCLUSION:**

Neither CD4 count nor HIV viral load at baseline predicted prognosis or survival; however there was a borderline significance of CD4 ( $P=0.058$ ) towards a better survival.

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Both CD4 count and HIV viral load were significantly changed over time that might be attributed to therapeutic effect.

Simple hematological (WBCs & lymphocytes) and biochemical (GGT& ALP) tests can be useful in predicting therapeutic response and survival in patients with AIDS-KS especially where resources are limited.

Further studies with a larger sample size and longer period of evaluation are recommended to evaluate these parameters in order to establish clearer guidelines for evaluation of patients with HIV-KS in South Africa.

## **CHAPTER 1**

### **1. HIV Epidemic**

Cancer and AIDS are both pandemics: they both cause psychological, emotional and social disruption that influence life as much as their physical impact [1]. The HIV epidemic was first identified in 1981 in homosexual males in Los Angeles and New York where atypical *Pneumocystis carinii* pneumonia cases were noted. This was found to be related to an immune deficiency, which had additional clinical consequences, including other forms of opportunistic infection; Kaposi's sarcoma and other malignancies. Kaposi's sarcoma is an AIDS defining malignancy in the presence of a positive HIV test [1].

#### **1.1 HIV and AIDS Epidemic in the world**

There are 42 million people living with HIV/AIDS worldwide. 38.6 million of these are adults; 19.2 million are women and 3.2 million are children under the age of 15. In 2002 five million people became infected with HIV, of which 4.2 million were adults and 2 million of them were women. HIV-related causes resulted in 3.1 million deaths in the year 2002, another new 5 million people became infected with HIV in 2003, this was the highest number since HIV was identified as an epidemic. At the global level, the number of people living with HIV continue to grow. Over 20 million have died as a consequence of HIV infection since AIDS was identified in 1981. The total number of people living with the human immunodeficiency virus (HIV) rose in 2004 to reach its highest level: an estimated 39.4 million [35 million– 44.3 million], Approximately 4.9 millions acquired HIV



infection in 2004 [2]. The estimated number of people living with HIV is shown in Figure 1.

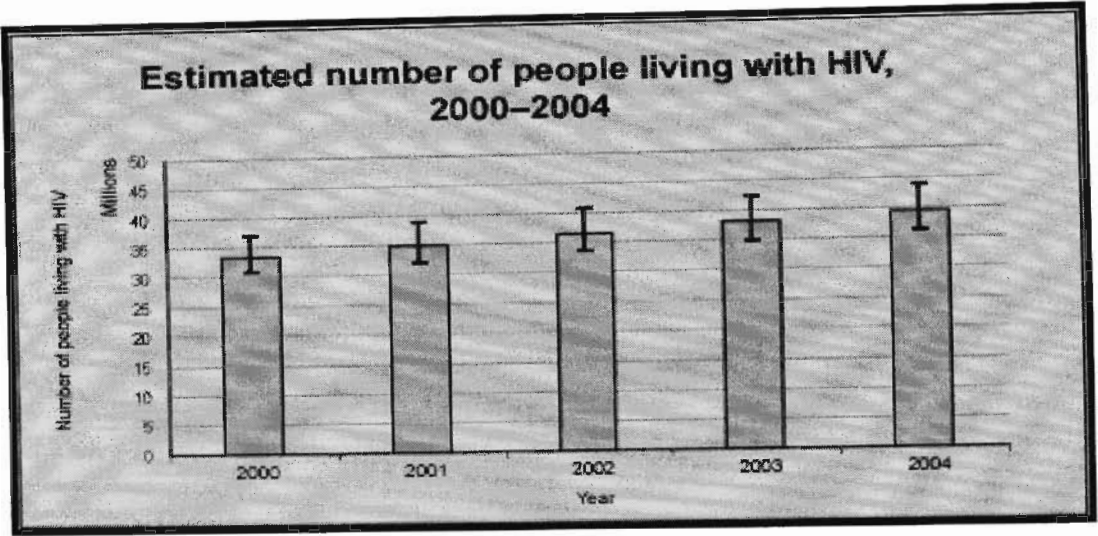


Figure 1: estimated number of people living with HIV 2002-2004 [3].

Sub-Saharan Africa has the highest prevalence of HIV (two thirds of people living with HIV/AIDS). South and South-East Asia has the second highest prevalence of HIV world wide (6 - 7 millions). There are 980,000 people living with HIV/AIDS in North America, 570,000 in Western Europe and 1.2 million in Eastern Europe and Central Asia. In Australia and New Zealand the number of HIV-infected people has remained constant since 2001 (15,000 people). Latin America and the Caribbean have 1.5 million and 440,000 HIV-infected individuals; respectively. East Asia and the Pacific have 1.2 million people living with HIV/AIDS. North Africa and the Middle East have 550,000 people living with HIV/AIDS [3]. The extent and impact of epidemic varies with different regions; some countries are more affected than others and within different areas of the same country, there are usually wide variations in infection levels between different provinces, states or districts [3].

## 1.2. HIV in Africa

An estimated 25 million people are living with HIV in Sub-Saharan Africa. Although there is an increase in AIDS-related deaths, prevalence rates remain steady due to continued increase in new infections. However, prevalence is still rising in some countries such as Madagascar and Swaziland, and is declining in others such as Uganda. Sub-Saharan Africa is a place of nearly two-thirds of all HIV-infected people. In 2003, an estimated three million people became newly infected and 2.2 million died. There is great diversity across the continent in the levels and trends of HIV infection. In southern Africa all seven countries have antenatal prevalence rates above 17% with Botswana and Swaziland having prevalence above 35%. In West Africa, HIV prevalence is much lower with no country having a prevalence above 10% and most having prevalence between one and five percent. Adult prevalence in countries in Central and East Africa falls somewhere between these two groups, ranging from 4% to 13%. African women are at greater risk, becoming infected at an earlier age than men. Today there are on average 13 infected women for every 10 infected men in sub-Saharan Africa – up from 12 for 10 in 2002. A review compared the ratio of young women living with HIV to young men living with HIV; showed a range of 20 women for every 10 men in South Africa to 45 women for every 10 men in Kenya and Mali [3]. In North Africa and the Middle East, around 480 000 are living with HIV but systematic surveillance of the epidemic is not well developed, particularly among high-risk groups such as drug users and homosexual men [3].

### **1.3. WOMEN AND AIDS**

The HIV epidemic is affecting women in increasing numbers; a global overview of the HIV-epidemic showed that approximately half of all people living with HIV are females. Women are becoming affected in an increasing proportion in regions where heterosexual sex is a dominant mode of HIV transmission, as is the case in sub-Saharan Africa and the Caribbean. In sub-Saharan Africa 57% of HIV-infected adults are women. In South Africa, Zambia and Zimbabwe, for example, young women (aged 15-24 years) are three to six times more likely to be infected than young men [3].

| Region                        | Adults & Children Living with HIV/AIDS* | Adult Infection Rate (%) | Deaths of adults and children* |
|-------------------------------|---|--------------------------|--------------------------------|
| Sub-Saharan Africa            | 25                                      | 7.5                      | 2.2                            |
| East Asia                     | 0.9                                     | 0.1                      | 0.04                           |
| Oceania                       | 0.03                                    | 0.2                      | 0.0007                         |
| South & South-East Asia       | 6.5                                     | 0.6                      | 0.46                           |
| Eastern Europe & Central Asia | 1.3                                     | 0.6                      | 0.049                          |
| Western Europe                | 0.58                                    | 0.3                      | 0.006                          |
| North Africa & Middle East    | 0.48                                    | 0.2                      | 0.024                          |
| North America                 | 1                                       | 0.6                      | 0.016                          |
| Caribbean                     | 0.43                                    | 2.3                      | 0.035                          |
| Latin America                 | 1.6                                     | 0.6                      | 0.084                          |
| Global Total                  | 37.8                                    | 1.1                      | 2.9                            |

Table 1: Regional Statistics for HIV & AIDS end of 2003 [3]  
\* Millions

| Regional HIV and AIDS statistics and features,<br>end 2002 and 2004 |  |  |                          |                                       |
|---|--|--|--------------------------|---------------------------------------|
|   | Adults and children<br>living with HIV | Adults and children<br>newly infected with HIV | Adult prevalence<br>(%)* | Adult and child deaths<br>due to AIDS |
| <b>Sub-Saharan Africa</b>   |  |  |                          |                                       |
| 2004  | 25.4 million<br>[23.4–28.4 million]    | 3.1 million<br>[2.7–3.8 million]               | 7.4<br>[6.9–8.3]         | 2.3 million<br>[2.1–2.6 million]      |
| 2002  | 24.4 million<br>[22.5–27.3 million]    | 2.9 million<br>[2.6–3.6 million]               | 7.5<br>[7.0–8.4]         | 2.1 million<br>[1.9–2.3 million]      |
| <b>North Africa and Middle East</b>                                 |  |  |                          |                                       |
| 2004  | 540 000<br>[230 000–1.5 million]       | 92 000<br>[34 000–350 000]                     | 0.3<br>[0.1–0.7]         | 28 000<br>[12 000–72 000]             |
| 2002  | 430 000<br>[180 000–1.2 million]       | 73 000<br>[21 000–300 000]                     | 0.2<br>[0.1–0.6]         | 20 000<br>[8300–53 000]               |
| <b>South and South-East Asia</b>                                    |  |  |                          |                                       |
| 2004  | 7.1 million<br>[4.4–10.6 million]      | 890 000<br>[480 000–2.0 million]               | 0.6<br>[0.4–0.9]         | 490 000<br>[300 000–750 000]          |
| 2002  | 6.4 million<br>[3.9–9.7 million]       | 820 000<br>[430 000–2.0 million]               | 0.6<br>[0.4–0.9]         | 430 000<br>[260 000–650 000]          |
| <b>East Asia</b>  |  |  |                          |                                       |
| 2004  | 1.1 million<br>[560 000–1.8 million]   | 290 000<br>[84 000–830 000]                    | 0.1<br>[0.1–0.2]         | 51 000<br>[25 000–86 000]             |
| 2002  | 760 000<br>[380 000–1.2 million]       | 120 000<br>[36 000–360 000]                    | 0.1<br>[0.1–0.2]         | 37 000<br>[18 000–63 000]             |
| <b>Oceania</b>  |  |  |                          |                                       |
| 2004  | 35 000<br>[25 000–48 000]              | 5000<br>[2100–13 000]                          | 0.2<br>[0.1–0.3]         | 700<br>[<1700]                        |
| 2002  | 28 000<br>[22 000–38 000]              | 3200<br>[1000–9600]                            | 0.2<br>[0.1–0.3]         | 500<br>[<1000]                        |
| <b>Latin America</b>  |  |  |                          |                                       |
| 2004  | 1.7 million<br>[1.3–2.2 million]       | 240 000<br>[170 000–430 000]                   | 0.6<br>[0.5–0.8]         | 95 000<br>[73 000–120 000]            |
| 2002  | 1.5 million<br>[1.1–2.0 million]       | 190 000<br>[140 000–320 000]                   | 0.6<br>[0.4–0.7]         | 74 000<br>[58 000–96 000]             |
| <b>Caribbean</b>  |  |  |                          |                                       |
| 2004  | 440 000<br>[270 000–780 000]           | 53 000<br>[27 000–140 000]                     | 2.3<br>[1.5–4.1]         | 36 000<br>[24 000–61 000]             |
| 2002  | 420 000<br>[260 000–740 000]           | 52 000<br>[26 000–140 000]                     | 2.3<br>[1.4–4.0]         | 33 000<br>[22 000–57 000]             |
| <b>Eastern Europe and Central Asia</b>                              |  |  |                          |                                       |
| 2004  | 1.4 million<br>[920 000–2.1 million]   | 210 000<br>[110 000–480 000]                   | 0.8<br>[0.5–1.2]         | 60 000<br>[39 000–87 000]             |
| 2002  | 1.0 million<br>[670 000–1.5 million]   | 190 000<br>[94 000–440 000]                    | 0.6<br>[0.4–0.8]         | 40 000<br>[27 000–58 000]             |
| <b>Western and Central Europe</b>                                   |  |  |                          |                                       |
| 2004  | 610 000<br>[480 000–760 000]           | 21 000<br>[14 000–38 000]                      | 0.3<br>[0.2–0.3]         | 6500<br>[<8500]                       |
| 2002  | 600 000<br>[470 000–750 000]           | 18 000<br>[13 000–35 000]                      | 0.3<br>[0.2–0.3]         | 6000<br>[<8000]                       |
| <b>North America</b>  |  |  |                          |                                       |
| 2004  | 1.0 million<br>[540 000–1.6 million]   | 44 000<br>[16 000–120 000]                     | 0.6<br>[0.3–1.0]         | 16 000<br>[8400–25 000]               |
| 2002  | 970 000<br>[500 000–1.6 million]       | 44 000<br>[16 000–120 000]                     | 0.6<br>[0.3–1.0]         | 16 000<br>[8400–25 000]               |
| <b>TOTAL</b>  |  |  |                          |                                       |
| 2004  | 39.4 million<br>[35.9–44.3 million]    | 4.9 million<br>[4.3–6.4 million]               | 1.1<br>[1.0–1.3]         | 3.1 million<br>[2.8–3.5 million]      |
| 2002  | 36.6 million<br>[33.3–41.1 million]    | 4.5 million<br>[3.9–6.2 million]               | 1.1<br>[1.0–1.2]         | 2.7 million<br>[2.5–3.1 million]      |

Table 2: Regional HIV and AIDS statistics 2002 &amp; 2004 [3].



| Regional HIV statistics and features for women,<br>end 2002 and 2004 |      |  |   |
|--|------|--|---|
|  |      | Number of women (15–49)<br>living with HIV | Percent of adults (15–49)<br>living with HIV who are women<br>(%) |
| Sub-Saharan Africa   | 2004 | 13.3 million [12.4–14.9 million]           | 57  |
|  | 2002 | 12.8 million [11.9–14.3 million]           | 57  |
| North Africa and Middle East   | 2004 | 250 000 [80 000–770 000]                   | 48  |
|  | 2002 | 200 000 [62 000–620 000]                   | 48  |
| South and South-East Asia  | 2004 | 2.1 million [1.3–3.1 million]              | 30  |
|  | 2002 | 1.8 million [1.1–2.7 million]              | 28  |
| East Asia  | 2004 | 250 000 [120 000–400 000]                  | 22  |
|  | 2002 | 160 000 [79 000–250 000]                   | 21  |
| Oceania  | 2004 | 7100 [4100–11 000]                         | 21  |
|  | 2002 | 5000 [3000–7500]                           | 18  |
| Latin America  | 2004 | 610 000 [470 000–790 000]                  | 36  |
|  | 2002 | 520 000 [390 000–690 000]                  | 35  |
| Caribbean  | 2004 | 210 000 [120 000–380 000]                  | 49  |
|  | 2002 | 190 000 [110 000–360 000]                  | 49  |
| Eastern Europe and Central Asia                                      | 2004 | 490 000 [310 000–710 000]                  | 34  |
|  | 2002 | 330 000 [220 000–480 000]                  | 33  |
| Western and Central Europe   | 2004 | 160 000 [120 000–200 000]                  | 25  |
|  | 2002 | 150 000 [110 000–190 000]                  | 25  |
| North America  | 2004 | 260 000 [140 000–410 000]                  | 25  |
|  | 2002 | 240 000 [120 000–390 000]                  | 25  |
| TOTAL  | 2004 | 17.6 million [16.3–19.5 million]           | 47  |
|  | 2002 | 16.4 million [15.2–18.2 million]           | 48  |

Table 3: Regional HIV and AIDS statistics for women 2002 &amp; 2004 [3].

## **2. Malignancy in Acquired Immunodeficiency Syndrome**

Immunodeficiency of multiple aetiologies is associated with an increased risk of malignancy. The risk is variable, dependent on the severity and extent of the immunologic abnormality, and is not restricted to those individuals considered to have a lack of immune reactivity. The combined presence of immune activation coupled with immunodeficiency may be the most conducive situation, for the appearance of immunodeficiency-related tumours [4].

Malignancies have been detected in approximately 40% of all patients with acquired immunodeficiency syndrome (AIDS) sometime during the course of their illness. These cancers have been both a primary cause of death in some patients and also a source of considerable morbidity [5]. The varieties of HIV-associated tumours are listed in Table 4 [6]. In general, the epithelial tumours commonly seen outside the context of HIV infection are not increased in incidence within the context of the AIDS epidemic. However, a relatively narrow range of malignancies occurs with increased frequency, in an association with secondary infectious processes. In particular, Kaposi's sarcoma (KS) has recently been linked to a member of the gamma herpes virus family, KS-associated herpes virus (KSHV) [6, 7]. This virus, also known as human herpes virus-8 (HHV-8), is also seen in a small subset of non-Hodgkin's lymphomas. A far larger proportion of the AIDS-related lymphomas (ARL) are associated with Epstein-Barr virus (EBV). The squamous cell neoplasia seen in AIDS is almost exclusively due to human papillomavirus (HPV) infection [6, 8, 9].

Several tumours are suspected to be of increased incidence, and accumulating evidence supports this view; these tumours include Hodgkin's disease (which is EBV-related in most cases of AIDS). Leiomyosarcomas are seen with increased frequency in children with HIV infection and are uniformly noted to have EBV present within them [10]. Interestingly, this tumour is not noted with increased frequency in adults with AIDS, and sporadic leiomyosarcomas are not EBV-related. Plasmacytoma appears to occur with increased frequency and has a number of interesting features in AIDS; it is the malignant transformation of a cell population that is commonly disturbed in HIV infection. Plasma cell overproduction of immunoglobulin is the most common serologic abnormality associated with HIV infection. Malignant plasmacytoma is occasionally seen and may be associated with anti-HIV-specific immunoglobulin-secreting tumours. Of note, there are also recent data that suggest that Kaposi Sarcoma herpes virus (KSHV) might participate in plasma cell dyscrasias [11], but it is unclear how this virus is involved in the plasmacytomas seen in AIDS. The incidence of AIDS-related malignancies appears to be altered in the era of protease inhibitor therapy for HIV-1. This is particularly notable in KS, where the number of patients requiring systemic therapy for this disease has markedly diminished. Similar observations have been made regarding the incidence of primary CNS lymphoma, which is generally a manifestation of end-stage AIDS. However, the frequency of systemic lymphoma in AIDS appears to be far more finely modulated. Definitive epidemiologic data regarding these changes are still in evolution.



| AIDS-associated neoplasms |                          |
|---------------------------|--------------------------|
| 1.                        | Kaposi's sarcoma         |
| 2.                        | Non-Hodgkin's lymphoma   |
| 3.                        | Squamous cell neoplasia  |
| 4.                        | Plasmacytoma             |
| 5.                        | Hodgkin's disease        |
| 6.                        | Leiomyoma/leiomyosarcoma |

Table 4: AIDS-associated neoplasms [adapted from 6]

### **3. KAPOSI'S SARCOMA**

#### **3.1 General introduction:**

Kaposi's sarcoma (KS) was described initially in 1872 by Hungarian dermatologist, Moritz Kaposi in 5 patients presenting with "sarcoma idiopathic multiple hemorrhagicum" [12]. From that time until the current human immunodeficiency virus (HIV) disease epidemic identified with the Acquired Immunodeficiency Syndrome (AIDS), KS remained a rare tumour. In 1912 Sternberg termed this disease Kaposi's sarcoma — now referred to as classical Kaposi's sarcoma, while most of the cases seen in Europe and North America have occurred in elderly men of Italian or Eastern European Jewish ancestry, the neoplasm also occurs in several other distinct populations: young black African adult males, young children, renal allograft recipients, and other patients receiving immunosuppressive therapy. The disseminated, fulminant form of KS associated with HIV disease is referred to as epidemic KS to distinguish it from the classic, African and transplant-related varieties of the neoplasm. In addition, KS has been identified in homosexual men apart from the HIV disease epidemic [13].

Although the histopathology of the different types of the Kaposi's tumour is essentially identical in all of these groups, the clinical manifestations and course of the disease differs dramatically [14]. A key piece to the dilemma of KS pathogenesis was the 1994 discovery of a gamma herpes virus, human herpes virus type 8 (HHV-8), also known as Kaposi's sarcoma herpes virus [15]. HHV-8 was identified in KS tissue biopsies from nearly all patients with classic, African,

transplant-related, and AIDS-associated KS, but was absent from non-involved tissue [16-19].

Kaposi Sarcoma is a spindle-cell tumour thought to be derived from endothelial cell lineage; this condition carries a variable clinical course ranging from minimal mucocutaneous disease to extensive organ involvement. Kaposi Sarcoma can occur in several different clinical settings.

### **3.2 Types of Kaposi's sarcoma**

#### **3.2.1 Classic Kaposi's sarcoma**

Classic KS is a rare disease, occurs more often in males, with a ratio of approximately 10 to 15 males to 1 female [20]. In North Americans and Europeans, the usual age at onset is between 50 and 70 years of age [20]. Patients usually present with single or multiple asymptomatic red, purple, or brown patch, plaque, or nodular skin lesion. The disease is often limited to lower extremities, especially involving the ankle and soles [20]. Classic Kaposi's sarcoma usually runs a relatively benign, indolent course for 10 to 15 years or more with slow enlargement of the original tumours and the gradual development of additional lesions. Lymphedema and venous stasis of the involved lower extremity are frequent complications. Systemic lesions can develop in long standing cases, along the gastrointestinal tract, lymph nodes, and other organs [20].

These visceral lesions are generally asymptomatic and are most often discovered only at autopsy, although clinically, gastrointestinal bleeding can occur. Up to one-

third of the patients with classic KS develop a second primary malignancy, most often non-Hodgkin's lymphoma [21-23].

### **3.2.2. African Kaposi's sarcoma**

Kaposi sarcoma was recognized as a relatively common neoplasm endemic in native populations in equatorial Africa in 1950, it comprised approximately 9% of all cancers seen in Ugandan males. African KS is observed as an indolent neoplasm identical to the classic disease seen in Europe and North America or as an aggressive disease with fungating and exophytic tumours that may invade the subcutaneous and surrounding tissue including the underlying bone. In Africa, both the indolent and locally more aggressive forms of KS occur with a male to female ratio comparable to that observed with the classic KS tumour seen in North America and Europe. However, patients in Africa are seen at younger ages than their European counterparts. Lymphadenopathic form of KS is also seen in Africa primarily in children (male: female ratio 3:1). In these cases, the generalized lymphadenopathy is frequently associated with visceral organ involvement. The prognosis is very poor, with a 100% fatality rate within 3 years [24, 25].

### **3.2.3 Immunosuppressive treatment-related Kaposi sarcoma**

Kaposi sarcoma in association with immunosuppression in a renal transplant patient was first described in 1969. Since then, a number of renal and other organ allograft recipients receiving immunosuppressive therapy (Prednisone and Azathioprine) have developed KS shortly after the onset of immunosuppressive therapy [26]. Immunosuppressed renal transplant recipients have a higher risk of

KS of 150 to 200 times higher than in the general population. The average time to develop KS after transplantation is about 16 months. Although the tumour in these iatrogenically immunosuppressed patients often remain localized to the skin, widespread dissemination with mucocutaneous or visceral organ involvement is common. In some cases, the KS tumours have regressed as a result of reduction or changes in immunosuppressive therapy. Clinical management of renal transplant patients who develop KS is difficult and requires a balance between the risk of death from generalized KS and the risk of graft rejection and complications of renal failure that may occur if the immunosuppressive therapy is discontinued [20,26].

### **3.2.4 Epidemic Kaposi sarcoma**

A fulminant and disseminated form of KS in young homosexual or bisexual men was first reported IN 1981 as part of an epidemic now known as AIDS [27]. The underlying immunologic deficiency that characterizes HIV disease is an acquired disorder of cell-mediated immune functions. This immunologic deficiency and immune dysregulation predisposes the host to a variety of opportunistic infections and unusual neoplasms, especially KS. HIV itself may play an indirect role in the development of KS [28]. Approximately 95% of all the cases of epidemic KS in the United States have been diagnosed in homosexual or bisexual men. In the past, approximately 26% of all homosexual males with HIV disease presented with, or eventually developed KS during the course of their illness. By comparison, less than 3% of all heterosexual intravenous drug users with HIV disease developed KS. The proportion of HIV disease patients with KS has steadily decreased since

the epidemic was first identified in 1981 [29]. About 48% of AIDS patients in 1981 had KS as their presenting AIDS diagnosis. By August 1987, the cumulative proportion of AIDS patients with KS had diminished to less than 20%. The introduction of highly active antiretroviral therapy (HAART) may delay or prevent the emergence of drug-resistant HIV strains, significantly decrease viral load, lead to increased survival, and lessen the risk of opportunistic infections [30, 31]. The use of HAART might possibly be related to the continuing decrease (12%) in the incidence of KS as an AIDS-defining illness. Epidemic KS is usually characterized by multifocal, widespread lesions at the onset of illness [32]. These lesions may involve the skin; oral mucosa; lymph nodes; and visceral organs, such as the gastrointestinal tract, lung, liver and spleen. Most patients with HIV disease who present with the mucocutaneous lesions of KS feel healthy and are usually free of systemic symptoms as compared to those patients with HIV disease that first develop an opportunistic infection. Sites of disease at presentation of epidemic KS are much more varied than those seen in the other types of this neoplasm. In an early report on the clinical manifestations of the disease, 49 patients were described; eight percent had no skin involvement, 27% had localized or fewer than 5 skin lesions and 63% had innumerable skin lesions widely distributed over the skin surface area. Sixty-one percent of the patients had generalized lymphadenopathy at the time of the first examination. Four of these patients who had generalized lymphadenopathy in the absence of skin lesions or detectable visceral organ involvement at the time of presentation were found to have biopsy-proven KS localized to the lymph nodes. In 45% of the patients studied, KS lesions were found in one or more sites along the gastrointestinal tract. Twenty-nine

percent of the patients had either unexplained fever or weight loss when first seen. While most patients present with skin disease, KS involvement of lymph nodes or the gastrointestinal tract may occasionally precede the appearance of the cutaneous lesions. Eventually, almost all patients with epidemic KS develop disseminated disease [20, 32].

Progression often proceeds in an orderly fashion from a few localized or widespread mucocutaneous lesions to more numerous and generalized skin disease with lymph node, gastrointestinal tract disease, and other organ involvement. Pleuropulmonary KS is an ominous sign usually occurring late in the course of the disease, especially in those patients whose death is directly attributed to KS. Most patients with epidemic KS die of one or more complicating opportunistic infections.

### **3.2.5 Non-epidemic Kaposi sarcoma**

There have been several reports documenting KS in homosexual men who persistently have no evidence of HIV infection. These patients have an indolent and cutaneous form of the disease, with new lesions appearing every few years. Lesions occur most commonly on the extremities and genitalia but can occur anywhere on the skin [33]. These cases may indicate the presence of causal factors, other than HIV, that homosexual men may be exposed to due to their lifestyle.

### 3.3 Aetiology & Pathogenesis:

Kaposi's sarcoma (KS) remains the most commonly diagnosed malignancy in HIV infected people. The aetiology of KS remains unknown but evidence suggests that the disease is promoted by the effects of immunosuppression and immune activation, possibly combined with a sexually transmissible infectious agent. The exact cause of AIDS-KS is presently unknown and causes appear to be multiple. Kaposi Sarcoma is now known to be associated with  $\gamma$ -2 herpes virus designated HHV-8, also known as Kaposi Sarcoma herpes virus (KSHV).

The pathogenesis of Kaposi's sarcoma is better understood since the identification of the novel human herpes virus 8 (HHV8) which can be found in all forms of KS. Viral oncogenesis and cytokine induced growth as well as some states of immunocompromise contribute to its development [34]. Various pro-inflammatory cytokine growth factors can stimulate the proliferation and growth of KS cells, these include tumor necrosis factor  $\alpha$ , interferon  $\gamma$ , fibroblast growth factor, and vascular endothelial growth factor (VEGF) resulting in a hyperplastic polyclonal lesions with predominant spindle cells derived from lymphoid endothelial tissue [34].

Several virally encoded genes – e.g., bcl-2, interleukin 6, Cyclin D, G-protein coupled receptor and interferon regulatory factors (IRF) provide key function on cellular proliferation. Cyclin D increases the proportion of the actively cycling cells, production of bcl-2 analogue (vbcl-2) and a protein (vFLIP) both of which will



prevent apoptosis; G- protein coupled receptor (GPCR) stimulates angiogenesis by producing angiogenic protein which is also inhibitory to macrophages [34].

### **3.4 HHV-8 its discovery and disease associations**

From the earliest days of the AIDS pandemic, it has been clear that patients with HIV disease are at increased risk for neoplastic events. AIDS patients are excessively at risk for developing KS (20,000 times that of the general population and 70 times that of other immunosuppressed populations) [35]. Early concepts of AIDS-KS biogenesis linked KS development primarily to HIV infection. The HIV genome was not found within KS tumour cells, so any involvement of HIV in KS tumorigenesis would have to be indirect. Evidence for such indirect involvement was advanced by studies showing that HIV-infected cells can produce extracellular factors that potentiate the growth of KS tumour cells in vitro [36, 37,38,39].

The search for a new virus in KS tumours was then motivated by epidemiologic studies that pointed to the involvement of a sexually transmitted factor other than HIV in KS tumorigenesis [40].

A major breakthrough occurred in 1994 when HHV8 or Kaposi Sarcoma Herpes virus (KSHV) was first identified by Chang, Moore, and their collaborators [41], on the basis of DNA sequences detected in tissues from patients of AIDS-KS. Human herpes virus 8 (HHV8) has also been identified in classic KS, endemic African KS, and transplant- related KS [42]. The technique based on the polymerase chain

reaction (PCR), identified 2 small fragments of DNA that were reproducibly present in AIDS-KS specimens but absent in most non-KS tissues. The nucleotide sequences of these 2 fragments revealed 2 gamma- (lymphotropic) herpesviruses, indicating that these fragments were derived from a novel herpes viral genome. These sequences are found in virtually all AIDS-KS tumours [43]; and are not found in most normal tissues derived from patients at low risk for KS. Subsequent work has shown that the sequences are also found in KS specimens from HIV-negative individuals [43 -44].

The spectrum of HHV-8-related diseases has not been completely described to date. In addition to KS, HHV-8 may also cause other tumours such as primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD). Human herpes virus 8 (HHV-8) recently has been associated with primary pulmonary hypertension in 2 small studies [45-47], while a primary HHV-8 infection syndrome" was described in immunocompetent children [48]. Improvements in molecular biologic techniques and increased understanding about the pathogenesis of HHV-8 infection may result in the description of additional human illnesses associated with HHV-8 infection.

### **3.5 The Epidemiology of HHV-8**

DNA Sequences of herpesvirus were identified in almost 100% of amplifiable samples from AIDS patients with Kaposi's sarcoma (KS) and 15% of non-KS tissue samples from AIDS patients. As HHV-8 cannot be cultivated readily, the diagnosis of infection with HHV-8 relies either on assessing the antibody response to

infection or on detecting viral nucleic acid in clinical specimens. Using various serologic assays, many studies have found that the seroprevalence of HHV-8 infection varies widely, from approximately 1-3% of blood donors and up to 35 % of homosexual men in North America to more than 70% in regions of Africa where HHV-8 is endemic. At least 85% of patients with Kaposi's sarcoma have antibodies to HHV-8. The prevalence of HHV-8 infection approximately corresponds to the prevalence of KS. A relatively high seroprevalence of Human herpes virus 8(HHV-8) has been described among injection drug users and women with multiple sexual partners, [49, 50]. HHV-8 seroprevalence also has been shown to be higher among family members of HHV-8-seropositive persons [51, 52]. The virus may also occasionally be transmitted vertically from mother to child. HHV-8 has been transmitted by renal allografts, and two kidney transplant recipients have subsequently developed Kaposi's sarcoma.

### **3.6 Virology, genome organization and molecular epidemiology of HHV8**

Significant advances have been made in the basic understanding of the virology of HHV-8 infection since its discovery. The entire HHV-8 genome has been sequenced and the structure of the virion has been established. The tissue tropism of the virus been elucidated. The genes and gene products associated with latency and lytic replication have been characterized. Human herpes virus 8 (HHV8) is the first human  $\gamma$ -herpesvirus and has a tropism for lymphocytes, endothelial cells, keratinocytes and possibly marrow stromal cells. The HHV-8 genome is housed in an icosahedral capsid of approximately 1200 angstroms in diameter with a typical herpesvirus envelope that consist of an amorphous tegument and a lipid bilayer

for a size of 120-150 nm [53, 54]. The genome is a linear, double-stranded DNA of about 165-170 kilobases in length [54]. The complete HHV-8 genome has sequence similarities to other  $\gamma$ -herpes viruses including, herpesvirus saimiri (HVS), Epstein - Barr virus (HHV-4) and murine  $\gamma$ - herpesvirus 68 (MHV68) [55]. The genome contains over 80 open reading frames arranged in a long unique region flanked by multiple 801bp terminal repeat units of high G+C content. The long unique region contains blocks of conserved genes found in most herpes viruses, interspersed with blocks of non-homologous genes that are specific for HHV-8 and related viruses [53]. HHV-8 proteins with recognizable homology to cellular proteins include: complement binding protein (ORF 4), IL6-like cytokine (ORF K2), three chemokines (ORF K4, ORF K4.1 and ORF K6), Bcl-2 (ORF 16) anti-apoptotic factor, interferon regulatory factor (ORF K9), D-type Cyclin (ORF 72), FLICE inhibitory protein (ORF K13), cell adhesion-like molecule (ORF K14) G-protein coupled receptor (ORF 74). HHV-8 has a number of genes such as ORF K12 (encodes the highly expressed transcript, kaposin), and ORF K1, a transmembrane protein that interacts with immunoreceptor kinases, which are likely to play a role in tumorigenesis [53]. Functional studies suggest that these pirated genes may help the virus to evade immune responses, prevent cell cycle shutdown and interrupt activation of apoptotic pathways. This strategy has been referred to as "molecular piracy" of host cell genes. HHV-8 encodes a number of immunomodulatory factors:

Fas signalling in (virus-infected) target cells are triggered by Fas receptor multimerization on binding with membrane-bound Fas-L. Subsequent recruitment of the adaptor molecule Fas-activated death domain (FADD) leads to upstream

caspase (caspase 8) autoactivation and release, leading to downstream effector caspase activation (caspase-3, -6, -7) & apoptosis. Death-receptor triggered apoptosis can be inhibited at several points: at the initiator stage by FLIP or in the amplification loop by bcl-2. HHV-8 v-FLIP blocks apoptosis in virus-infected cells. In addition, the virus also encodes a decoy (non-signalling) Fas receptor [53].

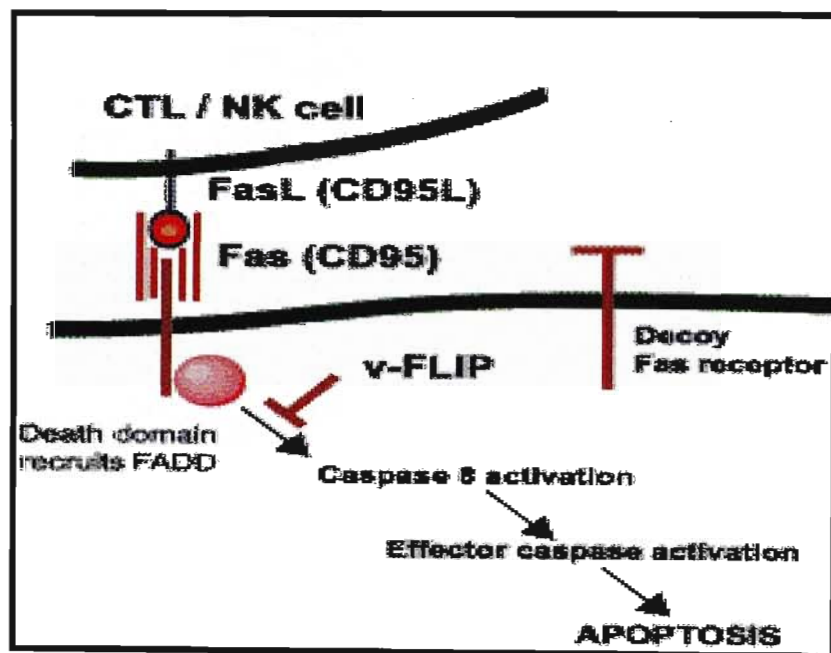


Figure 2: Molecular organization of HHV8 [adapted from 53]

Evidence suggests that one of the genes of HHV-8, vGPCR (viral G-protein coupled receptor) acts as a vascular switch, turning on synthesis of a powerful angiogenic agent, vascular endothelial growth factor (VEGF), which is responsible for the development of KS. However, HHV-8 also contains a considerable number of other 'pirated' cellular genes in an 'oncogenic cluster' within the virus genome which may also be involved in the development of malignancy, e.g. the K1 gene [53].

### **3.7 Sites of disease:**

Kaposi Sarcoma most commonly involves the skin; although involvement of the lymph nodes, the oral cavity, and gastrointestinal tract are often seen at presentation. Visceral lesions most commonly involve the stomach, bowel, liver, spleen, and lungs. Lesions are found in the GI tract in 40% of cases at diagnosis, up to 80% at autopsy, and are frequently a symptomatic. Pulmonary involvement, often occurring late in the disease, may cause severe respiratory symptoms and is associated with poor prognosis.

### **3.8 Staging classification of Kaposi's sarcoma:**

Several staging systems for KS have been proposed. A four-stage classification proposed by Krigel et al [57] was based entirely on tumour extent and was designed to include AIDS-associated KS as well as the "non-HIV epidemic" forms of the disease. A subsequent classification by Mitsuyasu, Groopman, and colleagues [58,59] designed specifically for AIDS-associated KS where patients are categorized according to extent of tumor and the presence or absence of systemic "B" symptoms and history of oral involvement (OI). Chachoua et al [60]; later proposed a classification that included the presence or absence of "B" symptoms and oral involvement (OI) and the CD4 count, but KS extent was not included as a staging variable.

Currently, the most widely used staging system is that proposed in 1988 by the Oncology Committee of the AIDS Clinical Trials Group (ACTG) [61]. This system takes tumor distribution, CD4 count, HIV-associated symptoms, and opportunistic

complications into account, and separates patients into good-risk and poor-risk groups for each of these three variables (Table 4). Subjecting this classification to prospective validation revealed each of the three variables to be significantly associated with survival [62]. In multivariate analysis, only the CD4 count and tumour extent were significantly associated with survival, however subsequent analyses suggest that a lower CD4 count (i.e. 150 cells/mm<sup>3</sup>) may be a better prognostic discriminate for survival than the originally proposed 200 cells/mm<sup>3</sup> cut-off. This study was performed on patients treated prior to the introduction of effective ART, and its current relevance has not been tested.

|                      | Good Risk (0) (Any of the following)  | Poor Risk (1) (Any of the following)  |
|----------------------|---|---|
| Tumour (T)           | Confined to skin and/or lymph nodes and/or minimal oral disease*                        | Tumour-associated oedema or ulceration<br>Extensive oral KS<br>Gastrointestinal KS<br>KS in other non-nodal viscera |
| Immune system (I)    | CD4 cells >= 150/mm <sup>3</sup>  | CD4 cells < 150/mm <sup>3</sup>   |
| Systemic illness (S) | No history of OI or thrush<br>No "B" symptoms #<br>Performance status >= 70 (Karnofsky) | History of OI and/or thrush<br>"B" symptoms present<br>Performance status <70                                       |

Table 5: ACTG Staging Classification for AIDS-Associated Kaposi's sarcoma

\* Minimal nodal disease is non-nodular KS confined to the palate.

# "B" symptoms are unexplained fever, night sweats, >10% involuntary weight loss, or diarrhoea persisting more than 2 weeks.

The above classification is applicable to: Classical Kaposi's sarcoma, Endemic Kaposi's sarcoma, Epidemic [AIDS-related] Kaposi's sarcoma With involvement of: cutaneous or muco-cutaneous structures, Lymph nodes or Viscera.

The revised CD4 cutoff of 150 cells/ $\mu$ L40 is lower than the original proposal of 200 cells/ $\mu$ L. However, in the HAART era, CD4 count do not appear to correlate with prognosis

### **3.9 Diagnosis:**

The diagnosis of KS in an individual known to be HIV positive is usually straight forward. The characteristic appearance of the cutaneous lesions in the form of pigmented macules, plaques, papules, or nodules that range in colour from brown or brown-red to reddish purple are sufficient to establish the diagnosis. They can range in size from a few millimetres to large confluent areas many centimetres in diameter. Lesions can involve any area of the skin but are common on the soles of the feet and rarely affects the palms of the hands.

Since the clinical appearance is so characteristic to the trained eye, biopsy can be reserved for those cases where there is clinical doubt. Biopsy confirmation establishes histological diagnosis. Suspected pulmonary lesions can be detected by bronchoscopy, but are generally not biopsied due to low yield and risk of haemorrhage. Chest x-rays and pulmonary gallium scans can also be used for evaluation. The concurrent presence of pulmonary infiltrates and nodular densities on chest x-ray, the lack of uptake of gallium in the pulmonary parenchyma and the presence of blood streaked sputum is virtually diagnostic for pulmonary KS when infectious causes have been ruled out. Kaposi Sarcoma involving the gastrointestinal tract is best diagnosed visually on endoscopy; biopsies are frequently negative because the lesions are sub-mucosal.



### **3.10 Treatment:**

The patient with AIDS-related malignancy presents two complex life-threatening diseases. Providing optimal treatment requires that the clinical team have a considerable understanding of HIV and the associated malignancy.

Therapeutic approaches of HIV-KS must take into account that there is no curative therapy, that the natural course of the disease may be quite variable and that a large numbers of systemic approaches have immunosuppressive effects. Many factors must be evaluated in developing a treatment strategy; the type of KS, the extent and location of the tumour, the organs involved, the presence of tumour-associated symptoms (e.g. pain, oedema, GI bleeding), symptoms of HIV infection, and the status of the patient's immune system. Kaposi Sarcoma may affect quality of life in a variety of ways; tumour-associated oedema may cause difficulty with ambulation; plaque-like or ulcerated cutaneous lesions may be painful, and bulky oral disease may interfere with speech or swallowing. In severe cases, bronchopulmonary involvement may lead to respiratory compromise, so the normal rules of palliative treatment should apply: in particular the concept that the treatment should not be worse than the disease itself. Variety of treatments have been used to treat HIV-KS and a range of responses been reported, however KS remains an incurable tumour.

Management for HIV-KS includes systemic and local treatment modalities. Systemic treatment includes chemotherapy, immunotherapy and antiviral drugs. Local treatment include, conventional and megavoltage radiotherapy, cryotherapy, intralesional chemotherapy and topical retinoids.

Although several chemotherapy agents have proven effective in controlling KS, the responses are often of limited duration and don't clearly improve survival. Neither local nor systemic treatments for AIDS-KS have been shown to prolong survival. Treatment with effective antiretroviral therapy (ART) has led to a dramatic decline in KS incidence. ART reduces the risk of KS by 50%. Anti-neoplastic treatment, in combination with ART that suppresses HIV replication, may produce prolonged remission of KS [63, 64]. Palliation of symptoms and improvement of quality of life remain major goals of treatment.

### **3.10.1 Antiretroviral therapy and Kaposi Sarcoma:**

The use of antiretroviral therapy started in the late 1980s; monotherapy with Zidovudine (azidothymidine, AZT) was shown to improve prognosis and clinical response in patients with HIV-related immunosuppression. This was attributed to improved immunity as reflected by reduced HIV viraemia and improved CD4 lymphocyte count [63].

Despite improvement in response, improved immunity was transient due to the emergence of therapy resistant new HIV strains [66]. In mid 1990s protease inhibitors were introduced and licensed for use.

Protease inhibitors act by blocking HIV replication for long periods of time. Clinical trials showed that the use of protease inhibitors combination was associated with improved survival and outcome [67].

Since the introduction of highly active antiretroviral therapy (HAART) in 1996 for HIV infection, the clinical outcomes for persons living with AIDS have improved significantly, and this includes those affected by neoplastic disease [68]. Highly active antiretroviral therapy has dramatically reduced the incidence of Kaposi's sarcoma, KS response rates have improved when HAART has been administered with chemotherapy [69]. The clinical response was attributed to improvements in immunity reflected by an increase in CD4 cell counts [70].

### **3.10.2 Systemic therapy:**

#### **3.10.2.1 Chemotherapy:**

Several cytotoxic chemotherapeutic agents were used and found to produce profound response with acceptable degree of side effects [71]. Chemotherapy can be used as a single agent or in combinations.

Agents such as vinca alkaloids (vincristine, vinblastine), bleomycin, doxorubicine, etoposide and topoisomerase inhibitors are active against HIV-KS. Chemotherapy is generally indicated in progressive, extensive cutaneous disease where rapid response is desired or in systemic visceral involvement. Several studies showed that the combined chemotherapy is highly effective in producing good clinical response and palliation of symptoms i.e. decreased oedema, decreased pain as well as improving systemic symptoms. The most frequently used combination chemotherapy regimens are doxorucin, vincrstine and bleomycin (ABV) and Bleomycin with vincristine (BV).

The doses of the commonly used chemotherapy agents are relatively high, doxorubicin 40 mg/m<sup>2</sup> every 4 weeks or 20 mg/m<sup>2</sup> every 2 weeks, vincristine 2 mg/m<sup>2</sup>, Bleomycin 10 I.U /m<sup>2</sup>. Cycles are repeated every 4 weeks. The ABV regimen was regarded as the standard of care for HIV-KS in the U.S. In South Africa, in the public sector and in most resource poor countries ABV has remained as an active, relatively tolerable and cost-effective regimen. However the treatment with conventional chemotherapy agents has been frequently limited with toxic effects and with the immunosuppressed status of HIV patients and their opportunistic infections, this led to the development of new chemotherapy approaches, the liposomal anthracyclines and paclitaxol [72].

### **Liposomal anthracyclines:**

A liposomal formulation of the chemotherapeutic agents is a new approach to a variety of malignant diseases. Liposomes are lipid and lipoprotein vesicles that encapsulate the active chemotherapy agent increasing their potential for targeting the tumour and thus reducing the toxic effects to normal tissues. The new formulation of anthracyclines results in longer plasma half life with smaller volume of distribution than the non-encapsulated form and higher drug concentration in KS tissue [73, 74]. Early clinical trials showed that Liposomal anthracyclines as a single agent chemotherapy has a clinical response equal or even better than ABV with less toxic effects [75].

Liposomal anthracyclines (Liposomal doxorubicin and daunorubicin) are currently considered as an initial first line treatment of AIDS-KS [76].

**Paclitaxol (Taxol)**

This chemotherapeutic agent has been developed and approved as second line therapy of AIDS-KS. Paclitaxol has shown substantial response rates and significant single agent activity both in patients with refractory tumours as well as in patients where paclitaxol used as first line treatment. Doses used are in the range of 100-135 mg/m<sup>2</sup> every 3 weeks.

**Investigational and experimental treatments of KS:**

Several investigational drugs for the treatment of Kaposi's sarcoma are under evaluation, these include antiangiogenesis compounds, cytokine inhibitors; signal transduction inhibitors, thalidomide and anti-HHV8 agents.

**Anti-angiogenesis Compounds:**

These investigational drugs act by inhibiting new blood vessel formation. Kaposi Sarcoma is highly vascular tumour with abundant amounts of VEGF. Inhibitors of VEGF are currently under evaluation and their use is restricted to clinical trials [77].

**Cytokine Inhibitors:**

Cytokines play a role in the pathogenesis of Kaposi Sarcoma; cytokines stimulate the growth and proliferation of KS cells. Targeted cytokine therapy is being evaluated; these include immunomodulation of some of the known cytokines such as IL-6, Bfgf, IL-6 monoclonal antibody. IL-4, downregulates IL-6 production and

retinoid compounds such as tretinoin. Interferon  $\alpha$  is used as a modulator of bFGF which reduces the production of bFGF and induces apoptosis [78].

### **3.10.2.2 Anti HHV8:**

Inhibition of HHV-8 can be suitable for both prevention and treatment of AIDS-KS. It has been shown that HHV-8 is sensitive to some antiviral drugs that prevent the replication of HHV-8. Sensitivity of HHV-8 to antiviral antibiotics ranges from high to cidofovir, moderate to ganciclovir and foscarnet and weak to acyclovir [79; 80].

### **3.10.2.3 Immunotherapy:**

Interferon  $\alpha$  has been in use as an active agent for localized skin KS lesions for more than two decades. Interferon has immunomodulation, antiviral and antiangiogenesis effects [81, 82]. A dose of 1-10million units/day is used; the dose is usually lowered when it is used with antiviral drugs to 1-5 million units/day. Interferon has shown better results when used with antiviral therapy [83].

### **3.10.3 Local therapy:**

#### **3.10.3.1. Topical retinoids**

As mentioned earlier Retinoid compounds act as immunomodulators of IL-6 which is involved in the pathogenesis of AIDS-KS. Retinoids induce a range of biological effects that include: cell growth inhibition, induction of normal cellular differentiation and initiation of apoptosis [84]. These effects are mediated through the binding of retinoid compound (Cis and Trans compounds) to intracellular retinoic acid receptors i.e., RAR- $\alpha$ , RAR- $\beta$ , RAR- $\gamma$  and retinoic acid X receptors RXR- $\alpha$ , RXR- $\beta$ , RXR- $\gamma$  [85].

The anti-proliferative and anti-tumour effects of retinoid compounds in vitro justify the rationale for their use in clinical trials [86, 87]. 9-cis-retinoic acid (alitretinoin [Panretin]) gel has received FDA approval for clinical use for treatment of localized cutaneous KS lesions.

#### **3.10.3.2 Radiation therapy**

Kaposi sarcoma is highly radiosensitive tumour; radiotherapy is considered the mainstay for palliative treatment of localized disease with extremely high rates of local control. Radiotherapy improves local symptoms of pain, oedema and bleeding. Excellent palliation of symptoms can be obtained with low energy electron therapy (6 MeV); doses of 800 cGy in single fraction, 2000-3000 cGy in 10 fractions can be used depending on site of lesions. Most superficial cutaneous lesions are treated with a large single fraction dose of 800 cGy. Lesions in other

areas like genital organs, hands and conjunctiva are treated with higher fractionated doses.

Since HAART was introduced for treatment of HIV, the natural history of HIV has changed significantly; this resulted in less practice of palliative radiotherapy and changes the trend and intention to more radical approaches.

### **3.10.3.3 Intralesional chemotherapy**

Intralesional injection of vinblastine showed relatively high response but for short duration [88, 89, 90].

### **3.10.3.4 Cryotherapy**

Liquid nitrogen was used primarily by dermatologists for palliative treatment of lesions of hands, face and neck with partial to complete response; cryotherapy can cause permanent hypopigmentation [91].

### **3.10.3.5 Surgical excision**

Although local surgical excision has been tried in certain sites such as oral cavity, shaft of the penis and eyelid lesions, however it doesn't seem to be reasonable as KS is a systemic disease.



#### 4. HIV and Kaposi sarcoma in South Africa:

South Africa faces a major health problem with HIV infection and its associated opportunistic infections and malignant diseases. South Africa has one of the highest and fast growing HIV epidemics in the world. It's estimated that there are approximately 5 million or 1 in 10 South Africans are now HIV positive, this accounts of about 20% of adults aged 15-49 year [92]. Examinations of women of childbearing age at antenatal clinics reveal a non-uniform geographical distribution throughout the country. The highest prevalence is in the province of Kwazulu Natal (35-40%); the lowest is in the Western Cape (7-8%); with intermediate levels in the other provinces (20-25%) [92].

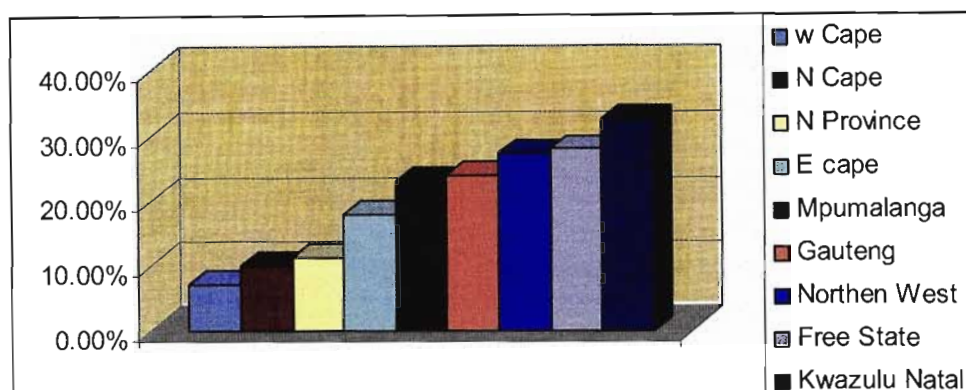


Figure 3: prevalence of HIV in South Africa

The impact on child health and infant mortality is also high, it's estimated that between 61,000- 89,000 newly infected children born annually to HIV-infected mothers; this contributes to higher childhood mortality rate.

HIV-related deaths account for about 40% of all deaths since HIV was recognized as an epidemic according to the medical research council (MRC) of South Africa, accordingly life expectancy fell by about 16.4% in the last 10-12 years – an

average of 53 years [92, 93]. Kaposi Sarcoma in South Africa was recognized as an endemic even before HIV, although the incidence of KS has risen up as the HIV epidemic grows [94]. Data from the cancer registry of South Africa showed that KS incidence was doubled and increased by seven folds in men and women respectively for the period 1992-1996 [95]. HHV8 is considered the major cause of HIV-KS. The prevalence of HHV8 is high in populations at high risk of KS i.e. HIV infected homosexual men and African population where KS has been endemic for long [96, 97, 98].

A recent study of African patients from Johannesburg and Soweto showed that HHV8 infection was strongly associated with KS [99]. Risk of KS was increased with increase antibody titer of HHV8, however for a given titer of HHV8 the risk was greater in HIV seropositive than seronegative subjects. It would seem that high HHV8 antibody titer reflects a high HHV8 viral load though only little data support this at the present time [99].

In three studies, it has been shown that seroprevalence of HHV8 in South Africa tend to increase with age and decrease with level of education. In the Johannesburg and Soweto study of black African patients the seroprevalence of HHV8 was slightly more than 30% compared with 20% of black blood donors and 5% in white blood donors. There was no sex variation [99, 100, 101]. The mode of transmission is still to be clarified, in South Africa there is weak evidence of sexual transmission as the prevalence of HHV8 is similar in individuals with or without HIV infection.

## **5. Evaluation of outcome:**

Despite all the advances in treatment, HIV-KS remains a major cause of morbidity and mortality in HIV infected individuals. Evaluation of outcome is measured in terms of clinical response and survival; however outcome is so far, determined by the degree and severity of immunosuppression (CD4 count) which seems to be inadequate for such assessment and needs to be reviewed with the new policy.

The average annual mortality rate of HIV-KS is approximately 40% and the median survival is 18 months from the time of diagnosis, taking into consideration that therapeutic interventions have far been suboptimal [102].

Unfortunately only little attention has been paid to the evaluation of outcome of HIV-KS and to the fact that the impact of the problem on survival is poor and the response is short-lived with the best palliative measures available.

In the year 2001, an audit of the patients with HIV-KS was done in the Oncology department at King Edward VIII hospital in Durban, South Africa. A total of 80 patients were evaluated for outcome, 42 males and 38 females. All patients had extensive mucocutaneous KS necessitating systemic therapy. Due to resource constraints; only 10/80 (12.5%) received chemotherapy according to oncology guidelines at that time. Of the remainder, 10/80 (12.5%) received no therapy at all and the majority, 60/80 (75%) patients were given palliative radiotherapy in a single dose to the area of greatest tumor burden. Only little symptomatic relief was obtained with the treatment approach mentioned.

As the treatment is considered suboptimal, we in Kwazulu-Natal have tried to establish a new strategy of definitive treatment with an appropriate, safe and effective therapeutic regimen. In view of this complexity and further more with the limited resources, providing treatment for all patients might not be an easy task. Hence the rationale for this study is to evaluate and identify several variables in a small cohort of patients over 6 months and to see if these variables can be predictive of response and survival. This is particularly relevant in South Africa, with the already high seroprevalence rates of HIV and HHV8, so that therapy and resources can be more appropriately directed. In the long term, these parameters may dictate a policy on selection criteria for therapy of patients with HIV-KS.

**Chapter 2****2.1 AIM**

The aim of the study is to identify which pre-treatment parameters were associated with therapeutic response in a group of patients with AIDS-associated Kaposi Sarcoma treated with HAART and chemotherapy and which of these parameters can be of prognostic value.

**2.2 OBJECTIVES**

1. To document clinical, haematological, immunological and biochemical parameters in all patients of HIV-KS at baseline and month 6 to be treated with chemotherapy.
2. To document outcome with respect to response to therapy and survival in all patients with HIV-KS who received standard chemotherapy and HAART at 6 months.
3. To evaluate which of these parameters is of prognostic value.
4. To assess the predictive value of change of certain parameters i.e. CD4, VL and correlate that with prognosis and response to treatment.

## **2.3 HYPOTHESIS**

Patients with AIDS-associated Kaposi sarcoma who present with low Haemoglobin and S.albumin and low counts of CD4, WBCs, Lymphocytes, neutrophils have poor prognosis and poor response to treatment.

## **2.4 METHODS**

This is an analysis of data from a prospective randomized controlled open-labelled phase III trial of patients with HIV-associated Kaposi sarcoma comparing HAART alone with the combination of HAART and chemotherapy. The study was approved by the research ethics committee and the postgraduate education committee of the University of KwaZulu-Natal. The study was performed at King Edward VIII hospital (KEH VIII) and Inkosi Albert Luthuli Central hospital (IALCH), two of major tertiary health care referral hospitals of the province of KwaZulu-Natal which has the highest prevalence of HIV infection throughout South Africa. Patients recruited for the study are seen at the Dermatology and Oncology clinics at KEH VIII and IALCH.

All patients referred to the study site are informed about the study; only those who gave informed consent were enrolled. In addition, consent was taken for photography in each patient. Patients were counselled about HIV test and have their lesions biopsied. Serological evidence of HIV and histologically confirmed Kaposi Sarcoma were required. Patients are staged into good risk and poor risk according to the AIDS clinical trial group (ACTG) criteria, which are based on extent of tumour (T),

the status of immune system (I) as measured by the count of CD4 cells and the extent of systemic illness (S), refer to chapter one for more details about ACTG staging system of AIDS-KS. Data from records of 25 patients who form part of this prospective trial were collected at base line (time at presentation) and again at 6 months with exception of demographic data that were taken only at presentation.

Data collected included:

- Epidemiological data: Age, Gender, Occupation, Ethnic group, Marital status, Level of education.
- Clinical data: Stage of disease according to ACTG.
- Haematological data: Haemoglobin, WBCs, Platelets, Lymphocytes, Neutrophils, Monocytes, Eosinophils and Basophils.
- Immunological data: CD4 count
- Biochemical data: Liver function tests, Kidney function.
- Viral data: plasma HIV serology and plasma HIV viral load

All of the 25 patients were treated with combination of HAART and standard Chemotherapy.

Treatment consists of:

**HAART:** one tablet daily of Triomune:

- Stavudine 40 mg BD for patients > 60 kg  
30 mg BD for patients < 60 kg
- Lamivudine 150 mg BD for patients >50kg  
2 mg/ kg for patients < 50 kg
- Nevirapine 200 mg BD  
(200 mg daily for first 2 weeks)

**Chemotherapy:** administered at 3 weekly intervals and consists of:

- Doxorubicin 20 mg / m<sup>2</sup> intravenously
- Bleomycin 10 U / m<sup>2</sup> intramuscularly
- Vincristine 1.4 mg / m<sup>2</sup> intravenously, maximum dose 2mg

Chemotherapy will continue for 2 cycles after maximum response unless toxicity developed. Maximum cumulative doses are 400 mg/ m<sup>2</sup> for Doxorubicin and 200 U/m<sup>2</sup> for Bleomycin.

At the initial visit 2.5 mls of venous blood were taken to confirm the HIV status of the patient. On the second visit 30 mls of venous blood were taken for haematological, chemical, immunological and virological studies, these included: FBC, LFT, U/E, CD4, HIV RNA Viral load. FBC and blood chemistries were repeated at 2 weekly intervals where 5 mls of blood were taken. At week 24, 30 mls of blood were taken for immunologic and virologic studies.



Tissue sample were taken at the initial visit for diagnostic purposes which was used for HIV-1 loads.

| WEEKS             | -2 | -1 | 0 | 2 | 4 | 6 | 12 | 24 | 48 |
|-------------------|----|----|---|---|---|---|----|----|----|
| HIV               | x  |    |   |   |   |   |    |    |    |
| PATHOLOGY         | x  |    |   |   |   |   |    |    |    |
| STAGING           |    |    | x |   |   |   |    |    |    |
| CLINICAL RESPONSE |    |    |   | x | x | x | x  | x  |    |
| FBC               |    | x  |   | x | x | x | x  | x  |    |
| CHEMISTRIES       |    | x  |   | x | x | x | x  | x  |    |
| CD4               |    | x  |   |   |   |   |    | x  |    |
| HIV RNA           |    | x  |   |   |   |   |    | x  |    |

Table 6: parameters and timing for patient assessment

**2.5 Definition of response**

Response to treatment was assessed by measurement of the above mentioned parameters at month 6 as per ACTG criteria, i.e. complete response, partial response, minimal response (stable disease) or disease progression.

Complete response (CR): absence of all evidence of disease and no appearance of new disease at least for 4 weeks.

Partial response (PR): reduction by at least 50% in the number of all previously existing lesions maintained for at least 4 weeks, with no new skin or oral or new visceral lesions.

Minimal response (stable disease): any response less than partial response or where neither PR nor PD criteria met.

Progressive disease (PD): more than 25% increase in one or more lesions or appearance of new lesions.

### **Statistical analysis:**

Data were analyzed in SPSS version 11.5 (SPSS Inc, Chicago, Ill, USA). Non-parametric statistics were used because of the small sample size and the skewness of the data. Variables were described using medians and ranges, and compared between two independent groups using Mann-Whitney tests. Baseline and 6 month comparisons were done using Wilcoxon signed ranks tests. ROC curves were used to analyze cut points to optimize sensitivity and specificity of a quantitative variable for a dichotomous outcome.

## **CHAPTER 3**

### **RESULTS**

#### **3.1 DEMOGRAPHY**

A total of twenty-five consecutive patients were evaluated over a period of 6 months at the dermatology clinic, King Edward VIII hospital as participants of a prospective randomized controlled open-labelled phase III trial of patients with HIV-associated Kaposi sarcoma comparing HAART alone with the combination of HAART and chemotherapy.

Of the twenty five patients 16(64%) were males and 9(36%) were females with a male to female ratio of 2.7:1. Ages ranged between 24 to 47 years with a median age of 34 years. All patients were of African ethnicity and Isizulu speaking.

With regard of education 4(16%) had primary school education, 17(68%) had secondary school education, 3(12%) had tertiary level education while 1(4%) was uneducated.

Nineteen (76%) were single, 3(12%) were married, 3(12%) were living together.

With respect of occupation, 1(4%) was a housewife, 11(44%) were labourers, 2(8%) were office workers, 1(4%) was a professional employee, 9(36%) were unemployed and 1(4%) whose occupation wasn't identified.

| <b>Gender</b>             | <b>No</b> | <b>%</b> |
|---------------------------|-----------|----------|
| Male                      | 19        | 64       |
| Female                    | 6         | 36       |
| Total                     | 25        | 100      |
| <b>Age</b>                |           |          |
| Median                    | 34        |          |
| Range                     | 25 - 47   |          |
| <b>Ethnic origin</b>      |           |          |
| African                   | 25        | 100      |
| <b>Occupation</b>         |           |          |
| House wife                | 1         | 4        |
| Labourer                  | 11        | 44       |
| Office worker             | 2         | 8        |
| Professional              | 1         | 4        |
| Unemployed                | 9         | 36       |
| Other                     | 1         | 4        |
| Total                     | 25        | 100      |
| <b>Marital status</b>     |           |          |
| Single                    | 19        | 76       |
| Married                   | 3         | 12       |
| Living together           | 3         | 12       |
| Total                     | 25        | 100      |
| <b>Home Language</b>      |           |          |
| Isizulu                   | 25        | 100      |
| <b>Level of education</b> |           |          |
| Primary                   | 4         | 16       |
| Secondary                 | 17        | 68       |
| Tertiary                  | 3         | 12       |
| None                      | 1         | 4        |
| Total                     | 25        | 100      |
| <b>Urban or Rural</b>     |           |          |
| Urban                     | 14        | 56       |
| Rural                     | 11        | 44       |
| Total                     | 25        | 100      |

Table 7: Demographic characteristics of 25patients on combination therapy

All patients were from the HAART and chemotherapy arm i.e. all were treated with the combination of HAART and chemotherapy.

All of the twenty-five (100%) patients had their haematological, biochemical, Virological and clinical parameters measured at base line. At month 6 only 22(88%) patients were alive, 3 (12%) patients died before 6 months, re-measurements of the same parameters was possible only for the 22 patients who remained alive.

### **3.2 Blood parameters**

Blood parameters were measured at baseline and month 6, the median values at base line were as follows: Hb 10.9 g/dl, WBCs  $5.95 \times 10^9/L$ , lymphocytes  $1.7 \times 10^9/L$ , neutrophils  $3 \times 10^9/L$ , platelets  $272 \times 10^9/L$ , S.albumin 30 g/L, total protein 88 g/L, ALP 64 U/L, and GTT 21 U/L. At month 6 the median values of the same parameters were, Hb 12.2 g/dl, WBCs  $4.65 \times 10^9/L$ , lymphocytes  $1.5 \times 10^9/L$ , neutrophils  $3 \times 10^9/L$ , platelets  $301 \times 10^9/L$ , S.albumin 36.5 g/L, total protein 84.5 g/L, ALP 78.5 U/L, GTT 44.5 U/L. Summary of all the values at baseline and month 6 is given table 8 below

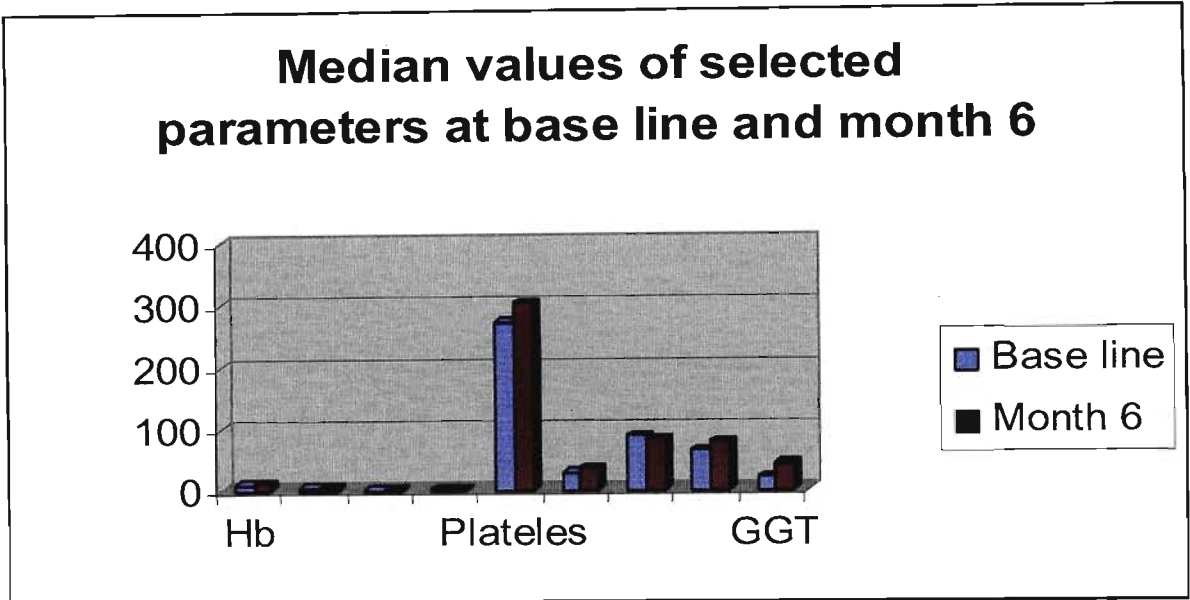


Figure 4: Baseline and month 6 blood parameters of patients on combination therapy

|             | Normal values                | Base line | Month 6 |
|-------------|------------------------------|-----------|---------|
| Hb          | 11.5- 15.5 g/dl              | 10.9      | 12.2    |
| WBC         | 4 – 11 x 10 <sup>9</sup> /L  | 5.95      | 4.65    |
| Lymphocytes | 20-45% x 10 <sup>9</sup> /L  | 1.7       | 1.5     |
| Neutrophils | 40-75% x 10 <sup>9</sup> /L  | 3         | 3       |
| Platelets   | 150-400 x 10 <sup>9</sup> /L | 272       | 301     |
| S.albumin   | 35-50gm/L                    | 30        | 36.5    |
| Protein     | 60-80gm/L                    | 88        | 84.5    |
| ALP         | 30-300iu/L                   | 64        | 78.5    |
| GGT         | 8-60 U/L                     | 21        | 44.5    |

Table 8: median values of different parameters at base line and month 6

### 3.3 Immunology

The median CD4 count was 255 cells/mm<sup>3</sup> (range 1-618) at baseline for the entire group of patients, at month 6 only 22 patients remained alive; their median CD4 count was 383.5 cells/mm<sup>3</sup> (range 84-1028), the median CD4 count for the 3 patients who died before month 6 was 47 cells/mm<sup>3</sup> (range 1 - 175). A change of CD4 count from baseline to month 6 was observed, median CD4 count was increased by 122.5 cells/mm<sup>3</sup> ( $p < 0.001$ ),

### 3.4 Virology

The median HIV-RNA viral load was 42000 (4.69 log<sub>10</sub>) (range 4700 to 720000) for the total number of patients at base line. For those who remained alive at month 6 the median viral load was 50500 (4.69 log<sub>10</sub>) (range 4700 to 720000). 3 patients died before month 6 for whom the median viral load was 31000 (4.5 log<sub>10</sub>) at baseline. VL decreased by a median of 2.88 logs from baseline to 6 months. At month 6 the median log viral load was 1.70, 90.9% of patients reached very low detectable or even undetectable levels of VLs ( $P < 0.001$ ).

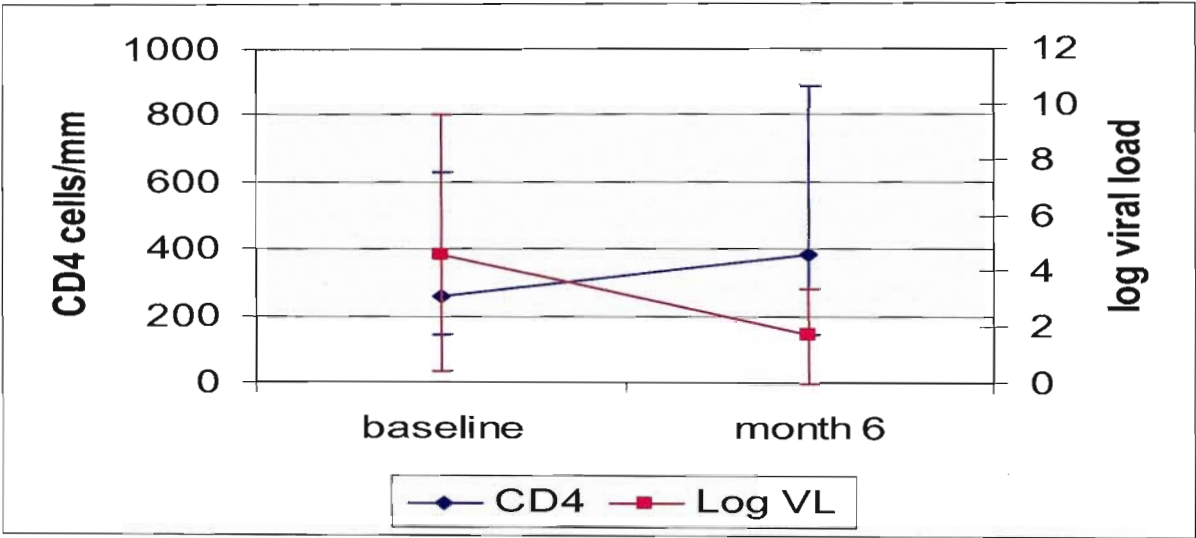


Figure 5: Figure demonstrating median CD4 recovery and viral load decline over a six month period (error bars show inter-quartile ranges)

**3.5 Therapeutic response**

21 (84%) patients were assessed for response as 4 (16%) patients did not reach month 6. Of the 21 patients 14 (66.6%) were males and 7 (33.3%) were females; 3 (14.3%) had complete response and 18(85.7%) had partial response. With respect to sex 2(14.3%) males had complete response and 12(85.7%) has partial response, 1(14.3%) female had complete response and 6 (85.7%) had partial response.



Figure 6a and 6b patient on combination chemotherapy and HAART before therapy



**Figure 6a**



**Figure 6b**



**Figure 7a**



**Figure 7b**

Figure 7a and 7b patient on combination chemotherapy and HAART after 6 months of therapy, demonstrating a partial response

3.6 Survival

3 patients died before month 6, their baseline haematological and immunological parameters are shown in the table below:

|         | Baseline haem | Baseline platelets | Baseline WBC | Baseline neutrophils | Baseline lymphocytes | Baseline monocytes | Baseline eosin | CD4B | log VL baseline |
|---------|---------------|--------------------|--------------|----------------------|----------------------|--------------------|----------------|------|-----------------|
| 1       | 12.3          | 324                | 3.8          | -                    | -                    | -                  | -              | 47   | 4.00            |
| 2       | 9.8           | 284                | 7.7          | 5.9                  | 1.0                  | .7                 | .10            | 175  | 4.78            |
| 3       | 9.2           | 113                | 6.8          | 2.6                  | 2.7                  | 1.3                | .20            | 1    | 4.49            |
| Total N | 3             | 3                  | 3            | 2                    | 2                    | 2                  | 2              | 3    | 3               |

Table 9: Baseline values for selected parameters for patients who demise before month 6

One patient was randomized to chemotherapy had low CD4 count and demised even before he received chemotherapy. Viral loads on HAART declined but CD4 did not recover.

Another patient received chemotherapy and HAART and developed liver impairment, after 2 months of therapy, most likely due to chemotherapy or Nevirapine.

The other patient was randomized to chemotherapy but due to very low CD4 count, did not receive it and demised due to severe immunosuppression and developed AIDS dementia terminally (Viral loads on HAART declined but CD4 did not recover).

3.7 Correlation with demographic factors

3.7.1 Age:

There was no significant difference in mean age between those who had a complete response (mean = 33.67 years) and those who had a partial response (mean = 34.72) ( $p = 0.758$ ).

|     | Overall response attained since entry/baseline | N  | Mean  | Std. Deviation | Std. Error Mean | P value |
|-----|--|----|-------|----------------|-----------------|---------|
| Age | Complete                                       | 3  | 33.67 | 8.505          | 4.910           | 0.758   |
|     | Partial response                               | 18 | 34.72 | 4.933          | 1.163           |         |

Table 10: Age – Response correlation in 21 patients

3.7.2 Gender:

Of the 21 patients who could be assessed for response, 2(14.3%) males had complete response and 12(85.7%) has partial response, 1(14.3%) female had complete response and 6 (85.7%) had partial response, i.e. males and females have exactly the same response rate with regard to complete and partial response. There was no association between gender and response rates ( $p = 1.000$ )

|       |        |              | Overall response attained since entry/baseline |                  | Total  |
|-------|--------|--------------|--|------------------|--------|
|       |        |              | Complete                                       | Partial response |        |
| Sex   | Male   | Count        | 2  | 12               | 14     |
|       |        | % within Sex | 14.3%  | 85.7%            | 100.0% |
|       | Female | Count        | 1  | 6                | 7      |
|       |        | % within Sex | 14.3%  | 85.7%            | 100.0% |
| Total |        | Count        | 3  | 18               | 21     |
|       |        | % within Sex | 14.3%  | 85.7%            | 100.0% |

Table 11: Sex \* Overall response attained since entry/baseline Cross tabulation

Chi-Square Tests

|                              | Value   | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|------------------------------|---------|----|-----------------------|----------------------|----------------------|
| Pearson Chi-Square           | .000(b) | 1  | 1.000                 | 1.000                | .726                 |
| Continuity Correction (a)    | .000    | 1  | 1.000                 |                      |                      |
| Likelihood Ratio             | .000    | 1  | 1.000                 |                      |                      |
| Fisher's Exact Test          |         |    |                       |                      |                      |
| Linear-by-Linear Association | .000    | 1  | 1.000                 |                      |                      |
| N of Valid Cases             | 21      |    |                       |                      |                      |

a Computed only for a 2x2 table

b 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.00.

3.7.3 ACTG staging

Of the 21 patients, 15 (71.4%) were of good prognosis and 6(28.6%) were of poor prognosis, 3(14.3%) had complete response and 18(85.7%) had partial response. The complete response in the good prognosis group was 20 % higher than the poor prognosis group, however wasn't statistically significant (p=0.526). The poor prognosis group was significantly associated with deaths by 33.3 % higher than the good prognosis group (p =0.037). Odds ratio could not be calculated because there were no participants in the good prognosis group who died; hence the reporting in percentage rather than odds ratio.

|           |      |                    | Overall response attained since entry/baseline |                  | Total  |
|-----------|------|--------------------|--|------------------|--------|
|           |      |                    | Complete                                       | Partial response |        |
| Prognosis | poor | Count              | 0  | 6                | 6      |
|           |      | % within Prognosis | .0%  | 100.0%           | 100.0% |
| Total     | good | Count              | 3  | 12               | 15     |
|           |      | % within Prognosis | 20.0%  | 80.0%            | 100.0% |
|           |      | Count              | 3  | 18               | 21     |
|           |      | % within Prognosis | 14.3%  | 85.7%            | 100.0% |

Table 12: Prognosis \* Overall response attained since entry / baseline Cross tabulation

|           |      |                    | Died before 6 month visit |       | Total  |
|-----------|------|--------------------|---------------------------|-------|--------|
|           |      |                    | no                        | yes   |        |
| Prognosis | poor | Count              | 6                         | 3     | 9      |
|           |      | % within Prognosis | 66.7%                     | 33.3% | 100.0% |
|           | good | Count              | 16                        | 0     | 16     |
|           |      | % within Prognosis | 100.0%                    | .0%   | 100.0% |
| Total     |      | Count              | 22                        | 3     | 25     |
|           |      | % within Prognosis | 88.0%                     | 12.0% | 100.0% |

Fisher’s exact p value = 0.037

Table 13: Prognosis \* Died before 6 month visit Cross tabulation

Chi-Square Tests

|                              | Value    | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|------------------------------|----------|----|-----------------------|----------------------|----------------------|
| Pearson Chi-Square           | 6.061(b) | 1  | .014                  | .037                 | .037                 |
| Continuity Correction (a)    | 3.315    | 1  | .069                  |                      |                      |
| Likelihood Ratio             | 6.889    | 1  | .009                  |                      |                      |
| Fisher's Exact Test          |          |    |                       |                      |                      |
| Linear-by-Linear Association | 5.818    | 1  | .016                  |                      |                      |
| N of Valid Cases             | 25       |    |                       |                      |                      |

a Computed only for a 2x2 table

b 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.08.

### **3.8 Correlation with blood parameters**

#### **3.8.1 Haemoglobin**

Comparison of baseline Hb between complete and partial response groups did not show any significance ( $p=0.580$ ) according to the statistical test applied (Mann-Whitney test).

#### **3.8.2 Total WCC and lymphocyte count**

Baseline WBCs and lymphocytes were significantly different for those who had complete response and those who had partial response with  $p$  values of 0.009 and 0.035 respectively.

WBCs showed 100% sensitivity and 93.3% specificity at a cut-off value of 9.7 while lymphocytes showed similar sensitivity and specificity at a cut-off value of 2.25.

#### **ROC Curves (Receiver Operating Characteristic curves):**

It is a graphical representation of the trade off between the false negative and false positive rates for different possible cut points of a diagnostic test, equivalently, the ROC curve is the representation of the tradeoffs between sensitivity ( $S_n$ ) and specificity ( $S_p$ ).

1. It shows the tradeoff between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity).
2. The closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate the test.
3. The closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test.
4. In this study, ROC curves were used to predict death and response using quantitative variables e.g. WCC, lymphocyte count, GGT and serum albumin.

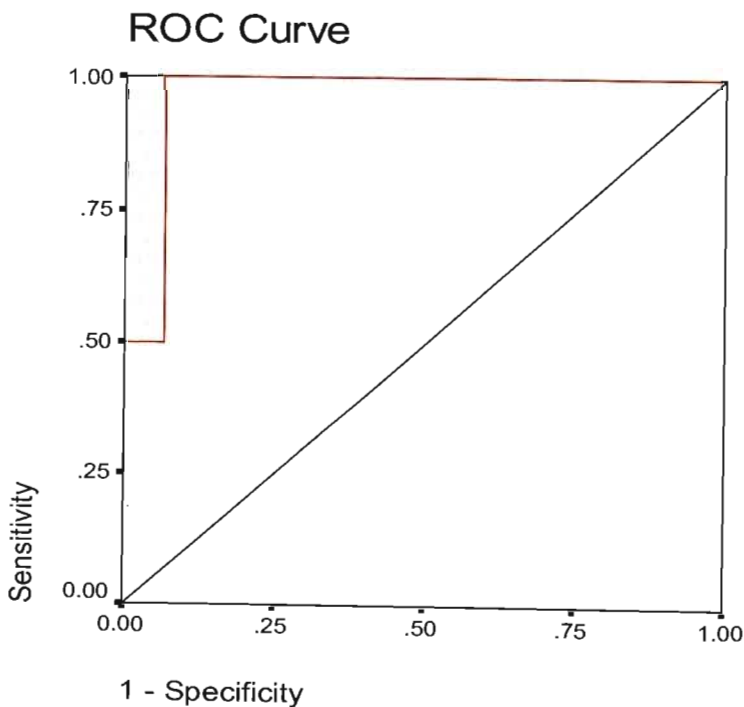


Figure 8: Baseline WBC ROC curve



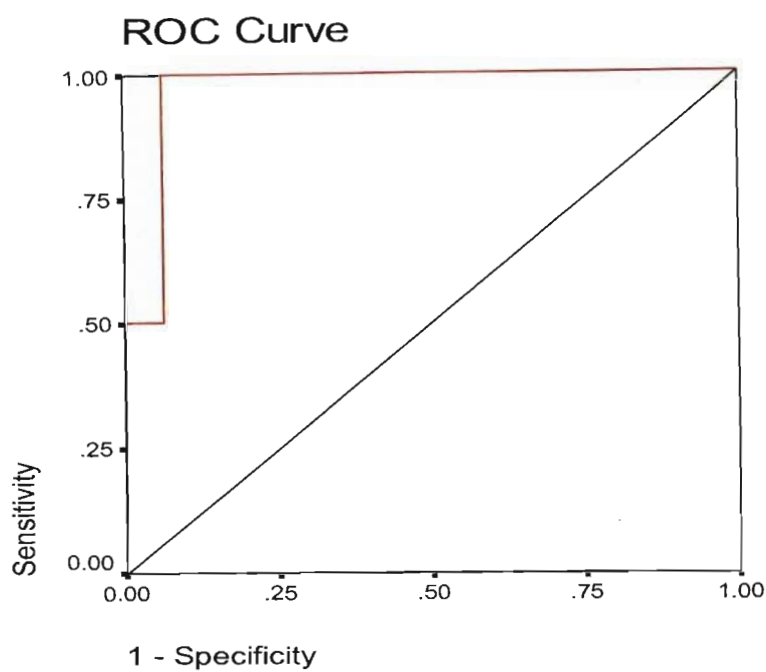


Figure 9: Baseline lymphocytes ROC curve

**3.8.3 Liver enzymes**

All blood parameters measured at base line were comparable for those who survived and who have died before month 6 with exception of ALP ( $P=0.006$ ) and Gamma GT ( $P=0.001$ ) which were much higher for those who died. GGT and ALP showed 100% sensitivity values of 70 and 84.5 respectively.

| Died before 6 month visit |         | N53ALKAL.1: 5.3<br>Alkaline phosphatase | N53GAMMA.1: 5.3<br>GGT |
|---------------------------|---------|---|------------------------|
| No 21                     | Median  | 59.50                                   | 20.00                  |
|                           | Minimum | 5                                       | 5                      |
|                           | Maximum | 115                                     | 69                     |
| Yes 3                     | Median  | 137.00                                  | 110.00                 |
|                           | Minimum | 90                                      | 71                     |
|                           | Maximum | 161                                     | 145                    |
| P value                   |         | 0.006                                   | 0.001                  |

Table 14: correlation of survival with ALP, GGT

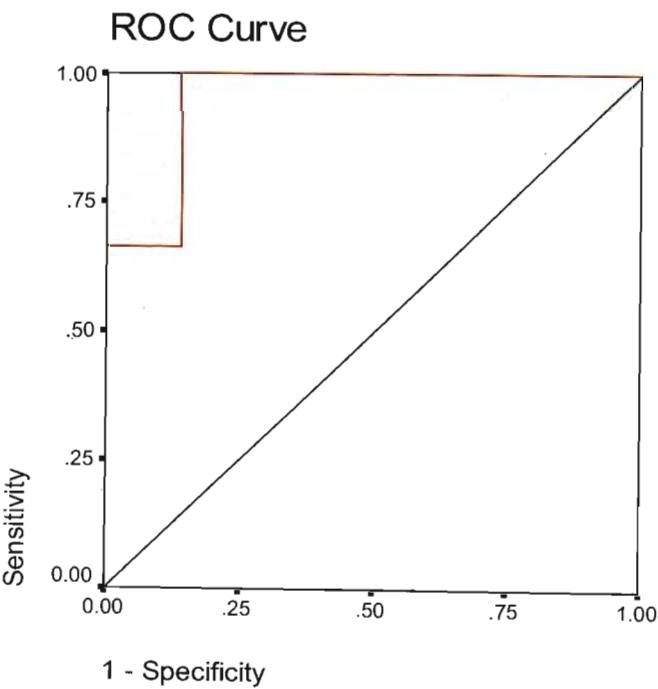


Figure 10: Baseline ALP ROC curve

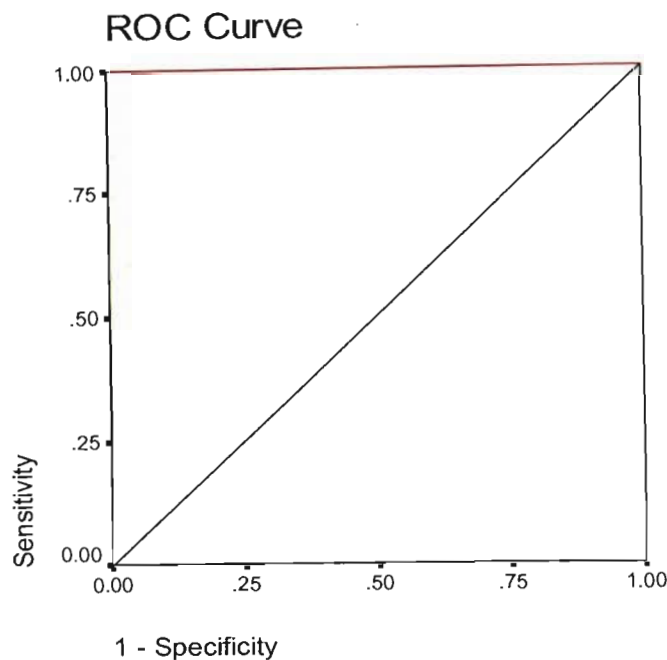


Figure 11: Baseline GTT ROC curve

**3.9 Correlation with CD4**

There was slight negative correlation between CD4 and log VL at baseline but it wasn't statistically significant (Spearman's rho=-0.115, p = 0.585).

Baseline CD4 count was higher in patients who remain alive than those who died at month 6, 288 cells/mm<sup>3</sup> and 47 cells/mm<sup>3</sup> for both groups respectively. (P=0.058) which considered as borderline significant. Baseline VL was higher in those who remained alive compared to those who died, although VL wasn't statistically significant to predict death (p = 0.446). This may be difficult to interpret but since the groups are not comparable it may be difficult to draw any conclusions from this observation.

**CHAPTER 4****DISCUSSION**

South Africa is one of the highest HIV-epidemic regions worldwide, it has the highest number of people living with HIV, the seroprevalence rate is approximately 20% of the total population. In Kwazulu-Natal where this study was conducted the prevalence of HIV is highest in the country and has been documented according to surveys in antenatal clinics to be 35-40%.

Kaposi Sarcoma is the commonest AIDS-associated cancer. People infected with HIV are 100 to 300 times more likely to have KS, consequently the HIV-related deaths were greatly increased and life expectancy markedly shortened. In South Africa HIV-related deaths account for approximately 40% of all deaths according to the Medical Research Council of South Africa [102].

In HIV patients, HIV-cancers present a complicated matter where general rules of cancer treatment don't apply. There are no well-defined rules or guidelines that standardize the treatment of HIV-related cancers in South Africa and in most provinces it is a departmental-driven protocol. These guidelines may be inappropriate and inadequate to provide control of the malignant disease. The treatment offered is usually the simplest, most cost-effective, palliative treatment available. Where HIV and cancer are both immuno-suppressant conditions, treatment of the cancer with

cytotoxic chemotherapy and radiotherapy will add to the already existing immunosuppressive status due to HIV, so control of HIV is an essential element to control cancer.

In our oncology department, the number of HIV-KS patients seen is quite large and they are almost always offered palliative radiotherapy, this is due to multiple factors: absence of specific guidelines, large number of patients, low CD4 count at presentation, high incidence of opportunistic infections and deaths and no antiretrovirals available in the past.

With the introduction of ARV therapy, all HIV patients will qualify and be eligible for ARVs; does this change the situation in South Africa?

This led to the question of whether or not we can establish a new policy or strategy of selection of those to be treated for HIV-KS. Hence the purpose of this study is to identify various hematological, biochemical, immunological, virological and clinical variables that can be of potential value as predictors of response and prognosis in HIV-KS. This is the first study of its kind on Black African patients with HIV-KS.

Unfortunately very few articles and little literature address the importance of these variables, however several retrospective studies evaluated some of these variables including age, gender, ethnic origin, CD4, CD8, plasma HIV load, p24 antigenaemia, IgA, IgG, site of KS lesions, concomitant opportunistic infections and some more,

nonetheless CD4 count and HIV viral load were most extensively studied. Evaluation of more variables is evolving.

In our study we directed our evaluation towards some basic routinely used blood parameters.

Our data analysis showed that age (mean= 34.52 years) and gender don't predict response; this is in contrast with Nasti et al [103], results which showed that KS at younger age was associated with more aggressive presentation, more severe immunodeficiency and more severe course in women, with shorter survival was seen more in women. In another study by the same author, survival wasn't influenced by age and sex. Most of other studies didn't show any association between age, and gender with response and prognosis. The association between ethnicity and response or prgnosis couldn't be assessed as all patients were of a similar ethnic origin.

All hematological and biochemical blood parameters including Hb, WBCs, lymphocytes, neutrophis, Monocytes, basophile, S.albumin, total protein, liver enzymes, bilirubin and renal functions were measured at baseline and month 6.

There is a dearth of such information in the literature, with an exception of one study by Spano JP et al [104], who retrospectively studied 78 HIV-infected patients; diagnosed as having KS between 1989 and 1995 with a median follow up of 22 months, Spano JP, concluded that high neutrophil count and a high serum

immunoglobulin A and G levels are independent predictors of prognosis. We found, in our 25 patients that the total neutrophil count was not predictive of response.

The rest of blood parameters such as Hb, platelets and S.albumin were not statistically significant to predict response.

In this study most of blood variables measured where comparable for those who survived and those who died before 6 months with exception of ALP ( $P=0.006$ ) and Gamma GT ( $P=0.001$ ) which were higher in those who died. The rest of liver parameters i.e. total protein, bilirubin, S.albumin and ALT were within normal limits for both those who died and who survived. This points toward the significance of Gamma GT and ALP as independent factors of survival. This is the first study to document the significance of liver enzymes as predictors of survival. Hence this has implications. Those with high GGT and ALP should be fully investigated before starting chemotherapy. These abnormalities may be due to a multitude of factors: Viral Hepatitis, TB, herbal use, alcohol or if it is not there at baseline and subsequently develops may be due to ARVs or due to the CXT itself, however hepatic infiltration by KS appear to be most likely cause, further imaging and biopsy would have elicited the most likely cause.

Baseline WBCs ( $p=0.004$ ) and lymphocytes ( $p=0.026$ ) were significantly higher in those with complete response, WBCs showed 100% sensitivity and 93.3% specificity at a cutoff value of 9.7 while lymphocytes showed similar sensitivity and specificity at

a cutoff value of 2.25, Gamma GT and ALP showed 100% sensitivity values of 70 and 84.5 respectively but with low specificity. This suggests that higher values of Gamma GT and ALP are associated with poor response and more deaths while WBCs and lymphocytes are highly sensitive measures and cutoff values can be used to predict response.

With respect to immunological and virological variables CD4 and HIV viral load were used to assess response, baseline CD4 was higher in those who has survived to month 6 with borderline significance value ( $p=0.058$ ). HIV viral load was higher in those who survived compared to those who died but wasn't statistically significant to predict survival. This is surprising and cannot be explained. There was slight negative correlation between CD4 count and log VL. At month 6 almost all patients showed very low or even undetectable levels of HIV VL, this is probably explained as a direct response to chemotherapy and HAART. Several studies showed diverse results with regard to CD4 count and VL, at the time Quinlivan et al [105], concluded that neither CD4 count nor HIV viral load predicted KS progression or KS clinical stage, Nasti et al [106] also concluded that CD4 count doesn't provide prognostic information. In contrast Hogg RS et al [107] demonstrated that low CD4 count ( $< 200$  cells/ mm<sup>3</sup>) was associated with KS progression and mortality, he concluded that there was low rates of disease progression and low mortality with CD4 counts of 200 cells/microL or higher as well with the use of PIs. Spano JP et al [104], results showed that low CD4



count was independently associated with shorter survival and best predicts over all survival.

Correlation of prognosis with CD4 changes over time doesn't show to be significant, as for both the good and poor prognosis groups the change in CD4 count was at a similar rate at baseline and at month 6; in other words categorizing patients as good and poor prognosis groups can't predict the rate of CD4 count increase.

Similarly correlation of HIV viral load with prognosis doesn't provide significant information i.e. there was no difference for good and poor prognosis in terms of VL changes over time, so again prognosis doesn't affect the rate of VL change. Although the change of CD4 ( $p=0.002$ ) and VL ( $p=0.000$ ) was significant over time which most likely is attributed to response to treatment, such change doesn't predict which patients will have either complete or partial response as there was no significant difference in CD4 and VL change between those who had complete or partial response.

There was a trend towards good prognosis associated with complete response; complete response was 20% higher in the good prognosis than the poor prognosis group, however the difference wasn't statistically significant ( $p=0.526$ ) probably due to the small sample size. Poor prognosis was associated with 33.3% ( $p=0.037$ ) more deaths than the good prognosis.

**Study limitations:**

1. Sample size is small but it's equally true that some of the variables may have reached statistical significance with a larger sample size.
2. Short follow up time of 6 months. Here again, we may have seen some variables unfold with respect to prognosis if the period of follow up had been longer.
3. Poor or uneven distribution of CR vs PR. Because there are so few with CR and most have PR, it is difficult to compare the groups.
4. This study was basically a prospective one, collected from data of a therapeutic trial. The initial study design and number of participants calculated was not powered or specific for a study addressing the prognostic indicators. A larger, well-designed study may be better able to address these issues.

**CHAPTER 5****CONCLUSION**

Epidemic AIDS-KS is the most common AIDS-associated malignancy. HIV patients who develop Kaposi Sarcoma present a complex situation. Palliative radiotherapy has been the corner stone for cutaneous and muco-cutaneous KS lesions, since chemotherapy in patients with AIDS, in the absence of antiretroviral therapy is riddled with problems. In the light of the national ARV rollout, there is a need to re-establish selection guidelines for therapy of HIV-KS.

Although our cohort is small, it questions the reliability of a variety of parameters as potential predictors of outcome and response in HIV-KS. Although it's difficult to predict from the initial presentation which patients are most likely to have a good response or a rapidly progressive tumors, our finding concluded that the use of simple, cheap, routinely used parameters such as WBCs ( $P=0.004$ ), lymphocytes ( $P=0.026$ ), ALP ( $P=0.06$ ), GGT ( $P=0.001$ ) appear to be of value to predict therapeutic response and survival where resources are limited. Whereas WBCs and lymphocytes were good predictors of response, ALP and GGT were associated with poor response.

Although CD4 and VL have been evaluated in many studies and results were diverse with respect of response and prognosis prediction, our findings showed that CD4 and

VL are poor predictors of prognosis and therapeutic response. These results can be used to define a new policy on selection criteria for therapy of patients with HIV-KS taking into account that access to this kind of therapy adds an extra burden on health care services.

Further studies with a larger sample size and longer period of evaluation are recommended to evaluate these parameters in order to establish clearer guidelines for evaluation of patients with HIV-KS in South Africa.

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